Universidade de Aveiro Departamento de Biologia
2016

Cristiana do Carmo Duarte Mendes

Avaliação Ecológica da Qualidade Ambiental de Sistemas Lóticos

Universidade de Aveiro Departamento de Biologia
2016

Cristiana do Carmo Duarte Mendes

Avaliação Ecológica da Qualidade Ambiental de Sistemas Lóticos

Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Biologia, realizada sob a orientação científica do Prof. Doutor Fernando Gonçalves, Professor Associado com Agregação do Departamento de Biologia da Universidade de Aveiro e co-orientação científica da Doutora Joana Luísa Pereira, Investigadora de Pós-Doutoramento no Departamento de Biologia e CESAM da Universidade de Aveiro.

Parte deste trabalho foi financiada por fundos europeus através do COMPETE e por fundos nacionais através da FCT, no âmbito do projeto PTDC/AACAMB/113515/2009."

Apoio financeiro do Instituto Politécnico de Viseu no âmbito da bolsa PROFAD – Universo II.

o júri

presidente

Doutor José Carlos Esteves Duarte Pedro
Professor Catedrático, Universidade de Aveiro

Doutor Amadeu Mortágua Velho Da Maia Soares
Professor Catedrático, Universidade de Aveiro

Doutor Diogo Francisco Caeiro Figueiredo
Professor Catedrático, Universidade de Évora

Doutor Fernando José Mendes Gonçalves
Professor Associado com Agregação, Universidade de Aveiro (orientador)

Doutora Anabela Clara Barreto Marques Novais
Professora Coordenadora, Escola Superior de Educação de Viseu

Doutor Ulisses Manuel Miranda Azeiteiro
Professor Auxiliar com Agregação, Universidade Aberta

agradecimentos

Chegada ao término desta tese de doutoramento, tenho plena consciência que estou mais enriquecida não só a nível científico, mas também, pela aproximação/ convivência com diversas pessoas, às quais não quero deixar de exprimir os meus sinceros agradecimentos.

Aos meus orientadores, Professor Doutor Fernando Gonçalves e Doutora Joana Luísa Pereira que aceitaram orientar-me neste trabalho e fizeram-no com toda a dedicação e espírito crítico. Agradeço-lhes também a amizade, o apoio, o incentivo, o contributo científico e os seus conselhos tão necessários à consecução deste trabalho, permitindo avanços significativos nos momentos mais difíceis.

Ao Instituto Politécnico de Viseu e à Escola Superior de Educação por me terem permitido a realização deste trabalho.

Um agradecimento enorme à Joana Santos pela a atenção que sempre me dispensou, pela valiosa e pronta ajuda, pelo seu saber e pelas palavras amigas.

À Ana Ré, à Tânia Vidal e ao Bruno Castro pela disponibilidade, colaboração no trabalho de campo, tratamento de dados e demais conhecimentos transmitidos.

À Inês e ao Sérgio, obrigada pelos momentos de alegria que me proporcionaram, os quais me davam mais força para continuar este trabalho.

Aos meus amigos e colegas da Escola Superior de Educação pela companhia e disponibilidade que sempre me demonstraram.

À Doutora Maria Paula Carvalho e à Doutora Anabela Novais, da Escola Superior de Educação de Viseu, agradeço a amizade, o carinho e a ajuda que me deram, de várias formas e em vários momentos deste percurso longo.

À minha família agradeço-lhes o carinho, o apoio e o incentivo que sempre me deram.

E finalmente, a todos os que, de maneiras tão diferentes tornaram possível e “escreveram” comigo esta tese, o meu MUITO OBRIGADA!

palavras-chave

Diretiva Quadro da Água, sistemas lóticos, avaliação do estado ecológico, comunidades de macroinvertebrados, Rio Sabor, Mina de S. Domingos, canais de Mira, impactos físicos (construção de barragens), impactos químicos (drenagem ácida mineira), impactos biológicos (espécie invasora - *Corbicula fluminea*), chave dicotómica.

resumo

A Diretiva Quadro da Água (DQA) estabelece um enquadramento para a ação comunitária no domínio da política da água e veio assim incumbir todos os Estados-Membros da União Europeia da proteção, melhoria e recuperação de todas as massas de água de superfície, com o objetivo final de alcançar um bom estado ecológico das mesmas. A importante inovação desta diretiva é, sem dúvida, a integração de abordagens físico-químicas, hidromorfológicas e biológicas para monitorizar e gerir o ambiente aquático. No entanto, a abordagem adotada pela DQA para a avaliação da qualidade biológica de massas de águas, é um pouco limitada à utilização de índices bióticos. Assim, o principal objetivo deste trabalho é avaliar a qualidade ecológica de sistemas lóticos selecionados, tendo em conta a forma como os diferentes agentes de stress (físicos, químicos e biológicos) afetam a estrutura das comunidades de macroinvertebrados bentónicos. Utilizando abordagens de análise abrangentes e diversas, em paralelo à linha metodológica e de análise de dados recomendada pela DQA, pretende-se discutir se estas últimas oferecem a resolução necessária para distinguir impactos diferentes da poluição difusa, para a qual esta diretiva terá sido definida. Os resultados obtidos evidenciaram que o uso de índices bióticos não era tão discriminativo como a análise multivariada da comunidade de macroinvertebrados. Esta última abordagem explora as tendências espaciais e temporais, permitindo uma análise mais detalhada da sucessão de espécies e também quantifica os fatores explicativos ambientais. O uso de macroinvertebrados bentónicos como indicadores do estado ecológico de sistemas lóticos pode ser vantajoso, mas as dificuldades na classificação taxonómica destes organismos pode restringir o acesso à metodologia pelo público não especializado. Neste sentido, uma chave de identificação, fotográfica, de macroinvertebrados existentes em Portugal, foi desenvolvida e validada. Os resultados demonstraram que a nova chave pode ser uma ferramenta útil para apoiar ações de educação ambiental, no âmbito da preservação dos ecossistemas lóticos.

keywords

Water Framework Directive, lotic systems, ecological status assessment, macroinvertebrate communities, Sabor River, S. Domingos mine, Mira channels complex, physical impacts (dams), chemical impacts (acid mine drainage) and biological impacts (*Corbicula fluminea* - invasive species).

abstract

The Water Framework Directive (WFD) established a holistic tool for Community action in the field of water policy, for all Member States of the European Union to protect, improve and restore all surface water bodies, with the ultimate goal of achieving good ecological status. The major innovation of this policy is undoubtedly the integration of physic-chemical, hydromorphological and biological approaches, to monitor and manage the aquatic environment. However, the approach adopted by the WFD for assessing the biological status of water bodies, is somewhat limited to the use of biotic indices. Thus, the main objective of this study is to assess the ecological quality of selected river systems, taking into account how the various stressors (physical and chemical) affect the structure of benthic macroinvertebrate communities. Using comprehensive and various analysis approaches in parallel with the methodological approach and data analysis recommended by the WFD, we intend to discuss whether the latter provide the necessary resolution to distinguish impacts that do not diffuse pollution, to which this policy has been set. The results showed that the use of biotic indices was not as discriminating as the multivariate analysis of the macroinvertebrate community. This latter approach explores the spatial and temporal trends, allowing a more detailed analysis of the succession of species and also quantifies the environmental explanatory factors. The use of benthic macroinvertebrates as indicators of the ecological status of lotic systems is advantageous in several ways, but difficulties in the taxonomical classification of these organisms may constrain the accessibility to the methodology by non-specialist audiences. In this context, a photographic (3D) identification key for freshwater macroinvertebrates found in Portuguese was developed and validated. Results showed that the new key can be a handy tool to support Environmental Education actions within the preservation of the lotic ecosystems.

ÍNDICE

CAPÍTULO I

Introdução Geral

1. ECOSSISTEMAS DULÇAQUÍCOLAS

A água é um recurso essencial à vida (Nebel & Wright, 1996), podendo ser adversamente afetado por agentes de origem antrópica (Ghate & Chaphekar, 2000). Este recurso encontra-se amplamente distribuído por todo o planeta (71 % da superfície do planeta é água). Do total da água existente, cerca de 97% é salgada e está confinada aos mares e oceanos (ecossistemas marinhos) (Connell, 2005) e a restante porção é água doce. A maior parte desta parcela encontra-se distribuída por cursos de água, lagos e aquíferos, que constituem os ecossistemas de água doce, dulçaquícolas ou limnéticos. Estes subdividem-se em lênticos (*lenis*, calma) ou de água parada (p.e x., lago, lagoa, charco ou pântano) e em lóticos (*lotus*, lavado) ou de água corrente (p. ex., nascente, ribeiro ou rio) (Odum, 2004).

A unicidade dos sistemas lóticos, comparativamente a outros, pode ser explicada pelo conjunto de vários fatores, nomeadamente (Giller & Malmqvist, 1998; Odum, 2004): i) a sua unidirecionalidade e forma linear (os rios atuam como canais de drenagem das águas superficiais, permitindo a ligação com o mar); ii) a existência de uma corrente definida e contínua, embora de velocidade muito variável em diferentes partes do mesmo curso de água, tanto longitudinal como transversalmente ao eixo da corrente, e de momento para momento; iii) a abertura do sistema, ou seja, a zona de contacto terra-água, é relativamente grande em proporção com o tamanho do sistema, o que significa que este está muito associado com a área circunvizinha para a obtenção de grande parte da respetiva energia básica. Para além disto, as comunidades que se organizam nos sistemas lóticos apresentam uma grande diversidade de espécies, com caraterísticas muito específicas em resultado de adaptações morfo-fisiológicas e comportamentais a este tipo de ambientes; as correntes que se geram, o tipo de substrato que transportam e a própria composição química da água são fatores que condicionam criticamente essas adaptações (Giller & Malmqvist, 2000). Assim, podem ser definidos quatro grandes grupos de organismos que compõem a rede funcional de um ecossistema lótico típico: microrganismos (decompositores de matéria orgânica - bactérias e fungos), vegetação lótica (produtores - macrófitas, briófitas e algas), macroinvertebrados (macroconsumidores - principalmente insetos) e vertebrados (macroconsumidores - peixes, anfíbios, répteis, aves e mamíferos) (Giller & Malmqvist, 2000) (Figura 1).

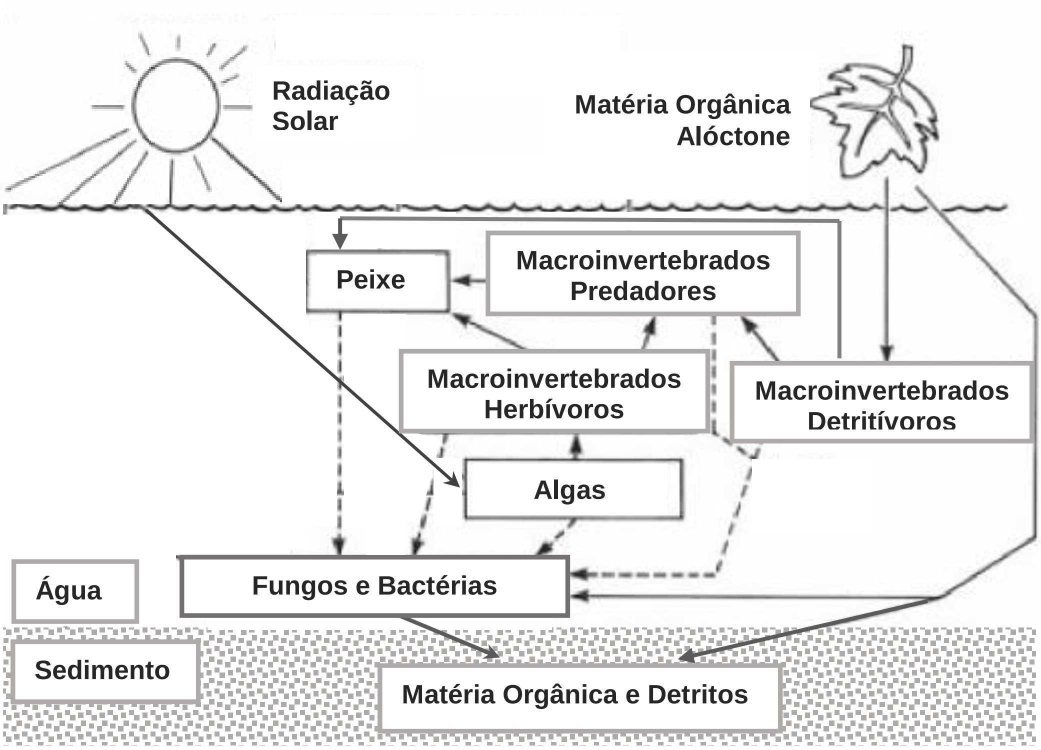


Fig. 1. Principais níveis funcionais presentes num ecossistema lótico típico, com a representação simplificada das respetivas relações tróficas (adaptado de Hellawell (1986)).

Para além do seu valor intrínseco enquanto ecossistemas de suporte à biodiversidade e recursos naturais *per se*, os ecossistemas lóticos são aproveitados pelo Homem para múltiplos fins, desde industriais (p. ex., produção de energia elétrica, captação de água para consumo, arrefecimento e processos de produção), agrícolas (p. ex., irrigação) a lazer (p. ex., parques temáticos, praias fluviais, desportos náuticos), entre outros. Todavia, a sua inadequada gestão e consequente degradação, assente no elevado aumento populacional e consequente crescimento económico e industrial, tem gerado inúmeros problemas que constituem uma preocupação por parte de muitos países (Ghate & Chaphekar, 2000). Um deles é a produção e transporte de contaminantes para as massas de água de superfície e em profundidade. Neste sentido, é reconhecido desde há várias décadas, que a contaminação e a poluição dos sistemas aquáticos devem-se não só a causas naturais (p. ex., atividade vulcânica e sísmica, fogos acidentais), mas também e, principalmente, a atividades antrópicas

(Crompton, 1997; Connell, 1999). Estas podem dever-se a descargas de efluentes domésticos (p. ex., detergentes, substâncias farmacológicas) e industriais e a fontes difusas provenientes da agricultura, que mantém o uso excessivo de fertilizantes e pesticidas (Varol, Gökot, Bekleyen, & Sen, 2012). As fontes difusas, em particular, podem proporcionar descargas de nutrientes nos cursos de água, acelerando o processo de eutrofização. Este fenómeno implica um aumento da produtividade primária, frequentemente caracterizada por uma proliferação de algas na zona pelágica que pode conduzir a uma depleção de oxigénio na coluna de água e na interface com o sedimento (Leaf & Chatterjee, 1999; Biggs, 2000; Nijboer & Verdonschot, 2004). Alguns dos problemas causados por esta situação incluem a degradação da estética (p. ex., ver Wharfe, Taylor, & Montgomery, 1984; Biggs, 1985; Biggs & Price, 1987; Whelch, Jacoby, Horner, & Seely, 1988), perda de invertebrados e de peixes por asfixia (p. ex., Quinn & Hickey, 1990) e degradação da qualidade da água (diminuição de oxigénio dissolvido e de pH) (p. ex., Quinn & Gilliland, 1989; Odum, 2004; Palaniappan et al., 2010).

Para além do cenário acima explorado, que constitui talvez a face mais visível da degradação dos ecossistemas aquáticos, há uma série de fatores mais específicos que podem refletir a pressão antrópica sobre os mesmos e contribuir para a geral degradação da sua qualidade que se tem vindo a observar a nível global (Palaniappan et al., 2010). No contexto da presente Tese, serão abordados três destes fatores específicos: a construção de barragens, a contaminação de cursos de água decorrente da exploração de minério metálico e a introdução de espécies invasoras.

1.1. *Construção de barragens*

As primeiras barragens foram construídas no antigo Egito, aproximadamente há 4000 a.C., e a partir do século XVI começou a verificar-se um aumento significativo do número destas infraestruturas (Kornijów, 2009). Desde 2000, o número de barragens aumentou, havendo mais de 800000 em funcionamento por todo o mundo (Xiaoyang, Shikiu, Qinghe, & Shiliang, 2010). Destas, cerca de 50000 são consideradas como grandes barragens (com mais de

15 metros de altura) e localizam-se, em maior número, na China, nos Estados Unidos da América, na Índia, no Japão e em Espanha (Kornijów, 2009). Nos últimos anos da segunda metade do século XX, a taxa de construção de barragens nos países desenvolvidos abrandou, provavelmente porque as zonas alagáveis tendem a ficar mais escassas (Marcé, Moreno-Ostos, García-Barcina, & Armengol, 2010). No entanto, um novo aumento na construção de barragens é quase inevitável devido a dependências futuras no abastecimento de água para o crescimento económico nos países em desenvolvimento (WCD - World Comission on Dams, 2000). Em Portugal, existem aproximadamente 231 barragens que obedecem ao critério de ter mais de 15 metros de altura, contados a partir das suas fundações, ou de a albufeira ter mais de 1 hm^3 de capacidade total de armazenamento (Agência Portuguesa do Ambiente, 2016). De entre estas, as mais recentes encontram-se no Norte de Portugal: Baixo Sabor (Trás-os Montes; 2008-2016), Ribeiradio Ermida (Sever do Vouga; 2010 - 2015), Foz Tua (Trás-os Montes; 2011-2016) e Fridão (Amarante; em licenciamento) (EDP, 2016).

De um modo geral, as barragens são construídas para múltiplos fins, como a produção hidroelétrica, irrigação e controlo de inundações (Xiaoyang et al., 2010). Estas infraestruturas podem beneficiar fortemente a exploração turística das zonas de albufeira, o que se traduz, muitas vezes, numa fonte de rendimento económico também significativo. Apesar destes e doutros benefícios para as populações humanas, as barragens apresentam, muitas vezes, consequências indesejadas tanto a nível local como regional, nomeadamente efeitos na conectividade hidrológica, na regulação da corrente, no transporte de sedimentos, na biodiversidade (Grill, Dallaire, Chouinard, Sindorf, & Lehner, 2014) e no *habitat* (Zganec, Duric, Hudina, & Gottstein, 2013).

Estima-se que, como resultado da construção de barragens, 59% dos 227 maiores rios do mundo foram fragmentados. Isto acarreta grandes mudanças no funcionamento dos ecossistemas lóticos e consequentemente na ecologia das suas comunidades (Nilsson, Reidy, Dynesius, & Revenga, 2005). Alterações na regulação da corrente, no transporte de sedimentos e na temperatura da água e sua oxigenação, provocam modificações na composição de espécies e na densidade de várias populações de plantas e animais dos rios (McCartney, 2009).

As barragens afetam a ecologia do sistema original através de modificações que operam tanto nos *habitats* terrestres (diminuição da área de *habitat* e alteração da sua configuração espacial) como nos *habitats* aquáticos (alteração da qualidade da água e modificação da morfologia do canal e da estrutura do leito do rio, aumentando o assoreamento a montante e erosão a jusante) (Xiaoyang et al., 2010; Vaikasas, Palaima, & Pliuraitè, 2013). As barragens criam barreiras físicas que impedem o fluxo natural da água, tendo impactos negativos no fluxo da biota a montante e a jusante do represamento da água como a degradação e fragmentação das populações (Vaikasas et al., 2013; Looy, Tormos, & Souchon, 2014). Por exemplo, Ziv, Baran, Nam, Rodríguez-Iturbe, & Levin (2012) demonstram o impacto negativo destas infraestruturas na sustentabilidade das populações de peixes, uma vez que estas são impedidas de migrar para montante. Um outro aspeto está relacionado com a má qualidade da água que ocorre, muitas vezes, em albufeiras, devido ao aquecimento da massa de água mais estagnada e ao enriquecimento em nutrientes (nitratos e fosfatos) (Gangloff, 2011), acelerando o processo de eutrofização. Todas estas alterações causam modificações na estrutura das comunidades de peixes e macroinvertebrados, através de mudanças na composição, abundância e diversidade tanto a montante como a jusante do represamento (Vaikasas et al., 2013).

As respostas dos macroinvertebrados a sistemas fluviais regulados são, muitas vezes, complexas e variáveis. No entanto, há evidências de que as condições hidráulicas são as forças motrizes que afetam a distribuição e abundância dos invertebrados bentónicos (Christopher, Granata, Murphy, & Livchak, 2007).

Estudos sobre macroinvertebrados bentónicos constatam, geralmente, que os locais de amostragem imediatamente a jusante das barragens são caracterizados por uma diversidade taxonómica menor do que os locais não afetados a montante, ou locais mais a jusante onde os efeitos da barragem se diluem (Céréghino, Cugny, P., & Lavandier, 2002; Camargo, Alonso, Puente, 2005). Marchant & Hehir (2002) mostraram que os locais a jusante de uma barragem abrigavam mais espécies tolerantes do que intolerantes. Devido à diminuição da taxa de fluxo da água, as barragens atuam como “armadilhas de depósito", em que os sedimentos finos ficam acumulados no interior do reservatório, alterando a estrutura do *habitat* e favorecendo a dominância de oligoquetas e quironomídeos (Kairo, Mols, Timm, Virro, & Jarvekulg, 2011), que são *taxa* considerados tolerantes a condições ambientais desfavoráveis.

Como já foi referido, mais de metade dos grandes rios do mundo apresentam barragens. No entanto, estudos sobre comunidades biológicas que comparem os sistemas lóticos, antes e após a construção de barragens, são relativamente escassos (Zganec et al., 2013). A falta de dados da situação ecológica antes da construção dificulta, seriamente, o trabalho urgente sobre a mitigação dos impactos negativos causados por estas estruturas (Malmqvist & Rundle, 2002).

1.2. *Exploração mineira*

Os recursos minerais têm sido explorados durante milhares de anos e a maioria dos desperdícios derivados desta atividade de exploração apresentam um impacto visual negativo na paisagem, devido ao volume de resíduos acumulados e à falta de vegetação (Santos, Abreu, Nabais, & Magalhães, 2012). Durante a extração do minério, vários resíduos (p. ex., escórias) ricos em metais ficaram expostos ao ar e foram responsáveis pela poluição dos solos, águas superficiais e sedimentos, devido, principalmente, à erosão e dispersão pelo vento (Harding, Quinn, & Hickey, 2000; MINEO, 2003; Smolders, Lock, Velde, Hoyos, & Roelofs, 2003).

A Europa foi uma das regiões mineiras mais importante do mundo e quase todos os países europeus apresentam vestígios de instalações mineiras. Embora a importância das atividades mineiras tenha diminuído na maioria dos países europeus, os locais abandonados permanecem e podem causar perigos ambientais (Wolkersdorfer & Bowell, 2005). Em Portugal, há evidências de exploração mineira desde os tempos pré-romanos, especialmente de Cu-Au-Ag e Fe-Cu-Sn. Na época romana explorou-se, principalmente, Au no norte do país, e Cu, Au e Ag na Faixa Piritosa Ibérica (FPI). Porém, a maior parte da atividade mineira ocorreu no século XX, com a exploração de depósitos de Fe e Mn, Sn e

W, de minérios radioativos como Ra e U, bem como depósitos de Ag e Au. Além disso, na FPI foram explorados depósitos de Cu e Sn, Cu, Zn e Pb, e pirite para S. Atualmente, apenas dois jazigos de metais estão ativos, Neves-Corvo (Cu, Sn) e Panasqueira (W, Sn). Ambos estão sujeitos a controlos ambientais, a requisitos de monitorização e medidas de remediação (Wolkersdorfer & Bowell, 2005). Das 175 minas abandonadas/desativadas (Tabela 1), algumas estão seriamente degradadas, apresentando grandes volumes de resíduos mineiros antigos e revelando um impacto ambiental negativo significativo (Wolkersdorfer & Bowell, 2005).

Tabela 1. Minas abandonadas existentes em Portugal (Wolkersdorfer & Bowell, 2005).

Tipo	Nº. de minas	Exemplos mais importantes
Minério radioativo	61	Urgeiriça, Quinta do Bispo, Cunha Baixa, Vale de Abrutiga, Castelejo, Bica.
Polimetálico	10	São Domingos, Aljustrel, Lousal, Caveira
Sn e W	40	Argozelo, Covas,Montesinho
Metais de base	28	Terramonte, Coval da Mó, Miguel Vacas
Fe e Mn	16	Orada, Cercal/ Rosalgar, Ferragudos
Carvão	3	São Pedro da Cova, Pejão
Au	12	Jales, Castromil, Penedono, Freixeda
Outros	5	Gouveia de Baixo, Cortes Pereira
Total	175	

Um estudo efetuado com 85 minas desativadas em Portugal revelou que 14% destas apresentam elevados níveis de perigo ambiental, mesmo após o seu encerramento (p. ex. minas de S: Domingos - Mértola) (Oliveira et al., 2002).

As minas desativadas, cujos resíduos se encontram a céu aberto e sem qualquer tipo de tratamento (Siwela, Nyathi, & Naik, 2010), podem ser, também, responsáveis por vários impactos nas áreas envolventes devido à natureza química dos seus efluentes, que continuam, na atualidade a ser emitidos para o ambiente (Lopes, Gonçalves, Soares, & Ribeiro, 1999). Estes efluentes consistem na drenagem ácida mineira (DAM), ou seja, material erodido dos resíduos provenientes da exploração das minas (Gomes, Antunes, Silva, Neiva, & Pacheco, 2010). Por exemplo, há formação de DAM quando as rochas que contêm sulfuretos (p. ex., pirite) são expostas ao oxigénio atmosférico durante a extração de metais (p. ex., Fe, Cu, Zn, Pb, As, U), do enxofre ou do carvão (Gray, 1998). Assim, na presença de água e de oxigénio, a oxidação, mediada por bactérias (p.ex., *Acidithiobacilus ferrooxidans*, *Leptospirilum ferrooxidans*), de rochas e minerais expostos (Hudson-Edwards, Jamieson, & Lottermoser, 2011) resulta na formação de um lixiviado altamente ácido e rico em metais e sulfatos (El Khalil, El Hamiani, Bitton, Ouazzani, & Boularbah, 2008).

Diversos estudos revelam que a DMA é capaz de promover impactos físico-químicos nos sistemas lóticos, nomeadamente, i) diminuição de pH, ii) aumento de concentrações de metais (p. ex., Fe, Zn, Cu, Al, Pb, As, Cd, Mn, Se) e de sulfatos e iii) formação de um precipitado estável de cor laranja conferida por oxi-hidróxidos ferro (Gray, 1996; DeNicola & Stapleton, 2002; Akcil & Koldas, 2006). Consequentemente, i) as comunidades biológicas ficam expostas a baixos níveis de pH e elevadas concentrações de elementos metálicos, o que, geralmente, conduz a uma diminuição da riqueza e diversidade da biota; ii) há alterações nos ciclos de nutrientes e alterações abióticas, como a destruição da capacidade tampão de bicarbonato, no sistema acidificado; iii) as comunidades biológicas tornam-se restritas a organismos tolerantes, que são capazes de sobreviver sob estas condições extremas (Rosenberg & Resh, 1993; Gray, 1997; Oberholster et al., 2013); e iv) ocorre perda da integridade dos ecossistemas, através da alteração da composição e dos atributos funcionais da fauna aquática (Svitok et al., 2014).

Os macroinvertebrados bentónicos são, geralmente, considerados como os indicadores mais sensíveis da contaminação por metais (Rosenberg & Resh, 1993), embora a resposta destes organismos a este tipo de contaminação possa variar em termos de abundância, diversidade e tolerância (DeNicola & Stapleton, 2002; Von der Ohe & Liess, 2004). A relação entre a DAM e a comunidade de macroinvertebrados dos rios tem sido amplamente estudada (p. ex., Roline, 1988; Malmqvist & Hoffsten, 1999; Battaglia, Hoose, Turak, & Warden, 2005), já que este grupo de organismos é, muitas vezes, reconhecido como um indicador da intensidade dos efeitos da DAM e utilizado para distinguir locais de referência e locais contaminados (Rosenberg & Resh, 1993; Kiffney & Clements, 1996;

Malmqvist & Hoffsten, 1999). Geralmente, a DAM é conhecida por modificar a composição de espécies e a diversidade de comunidades bentónicas e por diminuir a abundância e a biomassa em geral ou de um *taxa* específico (Gerhardt, De Bisthoven, & Soares, 2004; Van Damme, Hamel, Ayala, & Bervoits, 2008). Consequentemente, há alterações na estrutura trófica destas comunidades e nas teias alimentares existentes nos rios (Svitok et al., 2014).

Não há espécies indicadoras específicas para a DAM em rios contaminados, embora oligoquetas e dípteros (quironomídeos, em particular) sejam, geralmente, os grupos de macroinvertebrados mais encontrados a jusante da mina. Pelo contrário, os efemerópteros são mais sensíveis a ela, sendo o último grupo a recolonizar os rios após a contaminação (Gray, 1997). Os plecópteros são, também, considerados sensíveis a metais, enquanto os tricópteros são considerados mais tolerantes (Malmqvist & Hoffsten, 1999; Hickey & Golding, 2002).

Por causa do potencial risco ambiental da DAM sobre os ecossistemas dulçaquícolas e, especialmente, sobre os grupos da biota aquático mais sensíveis aos metais, como os efemerópteros (Malmqvist & Hoffsten, 1999), este agente de stress tem recebido uma atenção significativa.

1.3. *Introdução de espécies invasoras*

Espécies invasoras não indígenas são aquelas cuja distribuição se deslocou da sua área nativa, pela ação humana, de forma deliberada ou acidental, estabelecendo populações estáveis em áreas que anteriormente não estavam colonizadas (Strayer, 2012). O estabelecimento destas espécies foi identificado como uma das principais ameaças aos ecossistemas aquáticos, onde causam, em geral, perda de biodiversidade e impactos ambientais adversos (Pimentel, Zuniga, & Morrison, 2005; Hyytiainen, Lehtiniemi, Niemi, & Tikka, 2013). Estes impactos podem ser causados por interação direta com a comunidade residente (p. ex., competição, predação) e também por alterações indiretas nas condições do *habitat* (p. ex., turbidez, estrutura do *habitat*) (Gallardo, Clavero, Sanchez, & Vilà, 2016).

Classes de espécies invasoras, ecologicamente importantes em ecossistemas de água doce, incluem os peixes, em que os impactos mais evidentes foram perdas de presas privilegiadas, especialmente, nos casos onde o invasor não apresenta, no sistema, uma analogia trófica com a espécie nativa; os decápodes que atuam como poderosos omnívoros (utilizando como recursos alimentares algas, macrófitas, macroinvertebrados bentónicos, ovos de peixes), perturbando várias partes da cadeia trófica; plantas aquáticas, com um papel muito significativo no aumento descontrolado da biomassa vegetal e portanto da produção primária, afetando inclusivamente a qualidade dessa produtividade primária; e moluscos (Strayer, 2010). Estes últimos são uma das mais importantes classes de invasores. Sendo consumidores primários, alimentam-se de fitoplâncton e séston em suspensão na coluna de água, assim como, em alguns casos, de perifíton. Os moluscos podem desenvolver grandes populações em todos os tipos de águas doces, consumindo grande parte da produção primária. Consequentemente, afetam a quantidade e a composição dos produtores primários, perturbando assim toda a cadeia alimentar (Strayer, 2010). Os bivalves invasores, em particular, apresentam grande capacidade de criar, modificar e manter *habitats* e, também, de modelar a disponibilidade global dos recursos (Sousa, Gutiérrez & Aldridge, 2009; Illari, Souza, Antunes, Guilhermino, & Sousa, 2014). A modelação da disponibilidade de recursos resulta da grande capacidade de filtração que estes bivalves têm, diminuindo os recursos na coluna de água para outros organismos que se alimentem de material em suspensão e do aumento de nutrientes no sedimento lançados sob a forma de fezes ou de pseudofezes (Illari et al., 2012).

Dado que em Portugal ainda não há registo de avistamento de mexilhão-zebra, *Dreissena polymorpha* (Pallas, 1771) (DAISIE, 2006), o mais importante e problemático bivalve dulçaquícola invasor presente em bacias hidrográficas nacionais é a amêijoa asiática, *Corbicula fluminea* (O. F. Müller, 1774) (Rosa, Costa, Gonçalves, & Pereira, 2011). Esta espécie é considerada, atualmente, como uma das 100 piores espécies invasoras da Europa (DAISIE, 2008).

Trata-se de um bivalve de água doce oportunista que se dispersou a partir da sua área nativa no Sudeste da Ásia. Durante o século XX, esta espécie colonizou, primeiramente, Norte e Sul da América, antes de se estabelecer na Europa (Araújo, Moreno, & Ramos, 1993; Phelps, 1994; Darrigrand, 2002). As bacias hidrográficas portuguesas foram colonizadas, pelo menos desde a década de 80 (Mouthon, 1981), apesar de pescadores locais, do rio Tejo, assinalarem a presença destes indivíduos e os usarem como isco, no início de 1950. As principais bacias hidrográficas portuguesas, como por exemplo Minho, Lima, Douro, Vouga, Mondego, Tejo, Sado e Guadiana, foram colonizadas por *C. fluminea* (Rosa et al., 2011). A sua rápida dispersão envolve vetores de origem antrópica, como transporte nos cascos de barcos ou enredadas em macrófitas ou algas, comércio como isco ou acessório para aquários e transporte como curiosidade turística (Schmidlin & Baur, 2007; Sousa, Antunes, & Guilhermino, 2008a). A introdução de *C. fluminea* pode também dar-se naturalmente através do transporte de juvenis, pelas correntes de água, para jusante (p. ex., Mouthon, 2003; Hoyer, Schladow, & Rueda, 2015) e, eventualmente, para montante (Voelz, McArthur, & Rader, 1998) através da sua capacidade de flotação (Prezant & Chalermwat, 1984)

O seu sucesso invasivo deve-se a uma série de características biológicas sendo as mais relevantes um ciclo de vida rápido, produtivo e eficiente (Aldridge & McMahon, 1978; revisto em McMahon, 2002), um sistema reprodutivo com hermafroditismo associado à autofertilização opcional (Britton & Morton, 1982; Kraemer & Galloway, 1986), uma elevada competência ecológica, uma estreita associação com as atividades humanas, uma ampla variabilidade genética e plasticidade fenotípica, e uma grande capacidade filtradora (Sousa et al., 2008a).

C. fluminea é uma espécie particularmente problemática, já que causa alterações ecológicas nos sistemas invadidos, devido i) à competição pelo alimento com outras espécies filtradoras e/ou bentónicas, dada a sua elevada capacidade de filtração (Cohen, Dresler, Phillips, & Cory, 1984; Lauritsen, 1986; Strayer, 1999; Hakenkamp et al., 2001; Williams, Bunkley-Williams, Lilyestrom, & Ortiz-Corps, 2001; Schmidlin & Baur, 2007); ii) à ingestão de grandes quantidades de esperma, gloquídeos e juvenis de bivalves nativos (Strayer, 1999); iii) à produção de amónia e redução de oxigénio capazes de provocar a morte de bivalves nativos, aquando de episódios de mortalidades em massa, que são típicos nestes bivalves (Strayer, 1999; Sousa, Dias, Guilhermino, & Antunes, 2008b); iv) à alteração da dinâmica da matéria orgânica entre sedimento e coluna de água e consequente alteração dos ciclos de nutrientes (Hakenkamp & Palmer, 1999; Vaughn & Hakenkamp, 2001); e v) ao facto de serem vetores de parasitas e outros agentes patogénicos (Chung, Jung, Park, Hwang, & Soh, 2001). No entanto, esta espécie também apresenta aspetos positivos, nomeadamente i) as suas conchas podem fornecer abrigo e substrato para outras espécies, sobretudo quando ocorrem grandes acumulações após eventos típicos de mortalidade em massa (Strayer & Malcom, 2007; Werner & Rothhaupt, 2007); ii) fomentam a acumulação de matéria orgânica e nutrientes no substrato para espécies bentónicas (Vaughn & Hakenkamp, 2001; Cantanhede, Hahn, Gubiani, & Fugi, 2008; Sousa et al., 2008a); e iii) através da sua capacidade filtradora, podem apoiar a redução do nível de eutrofização da massa de água e diminuição da sua turbidez, permitindo o reaparecimento de vegetação aquática submersa (Phelps, 1994). No entanto, o enriquecimento do compartimento bentónico com matéria orgânica, a partir de fezes e pseudofezes, não é propriamente consensual (p. ex., Werner & Rothhaupt, 2007), devido à capacidade adicional da *C. fluminea* em se alimentar de matéria orgânica ressuspensa dos sedimentos, através dos movimentos do pé. Esta forma de alimentação reduz a quantidade de bactérias bentónicas e perifíton no sedimento (Hakenkamp & Palmer, 1999; Hakenkamp et al., 2001); portanto, as amêijoas podem utilizar a sua própria matéria orgânica depositada de forma eficiente, tornando-a indisponível para outros *taxa* bentónicos (Werner & Rothhaupt, 2007).

Os impactos ecológicos da amêijoa asiática *Corbicula fluminea* podem refletir-se numa mudança da qualidade ecológica de sistemas lóticos invadidos, que deve, como qualquer alteração resultante de qualquer outro agente de stress (p.ex., construção de barragens e contaminação por DAM, conforme contexto da presente Tese), ser detetável pelos sistemas de avaliação disponíveis. Estes são, naturalmente, regulados na legislação existente, sendo a Diretiva Quadro da Água a mais importante a este nível no contexto Europeu. É neste enquadramento que a identificação de impactos e a monitorização dos cursos de água assume importância vital, no sentido de promover as medidas mitigadoras e de reabilitação/restauração necessárias para obter o bom estado ecológico desejável e requerido para os sistemas aquáticos.

2. Diretiva Quadro da Água

Consciente de que 20% das águas superficiais da União Europeia (UE) estão sob um elevado risco de poluição e que metade das zonas húmidas está em perigo (CE, 2010), a Comissão Europeia publicou a Diretiva Quadro da Água (DQA) 2000/60/CE. O principal objetivo deste documento legal é o estabelecimento de um sistema para a proteção das águas de superfície interiores, das águas de transição, das águas costeiras e das águas subterrâneas (CE, 2000). Esta diretiva foi transposta para a ordem jurídica nacional através da Lei nº 58/2005, de 29 de Dezembro (Lei da Água) e do Decreto-Lei (DL) nº 77/2006, de 30 de Março. No geral, a DQA estabelece que os Estados-Membros protegerão, melhorarão e recuperarão todas as massas de águas de superfície, com o objetivo ambiental de que estas alcançassem um Bom Estado em 2015, ou seja, requer que as massas de água de superfície atinjam pelo menos o Bom Estado Ecológico e o Bom Estado Químico (INAG, 2009). No entanto, muitas massas de água da UE não alcançaram esta meta em 2015, apesar de se terem feito esforços e progressos no sentido do cumprimento do objetivo. As pressões hidromorfológicas, a poluição e as sobre-captações são as pressões mais significativas exercidas no meio aquático, reconhecidas como as que mais impedem a melhoria do estado das massas de água (CE 670, 2012). Além disso, cerca de 15% das massas de água de superfície existentes no território da UE estão num estado ecológico desconhecido e cerca de 40% num estado químico desconhecido. Em alguns Estados-Membros, desconhece-se o estado ecológico e químico de mais de 50% das massas de água. Por isso, a DQA permite que os Estados-Membros recorram a uma possibilidade de derrogação, com base nas condições naturais da massa de água, e adiem o prazo até 2027 (CE 670, 2012). Em Portugal, 52% das massas de água superficiais atingiram o “estado bom” em termos ecológicos e químicos, cerca de 45% estão abaixo disso e 2% não são classificáveis, por falta de dados (Garcia, 2015).

O estado ecológico traduz a qualidade estrutural e funcional dos ecossistemas aquáticos associados às águas de superfície, e é expresso com base no desvio relativamente às condições de uma massa de água semelhante, ou seja, do mesmo tipo, em condições consideradas de referência. O termo "tipologia" designa grupos de massas de água com características geográficas e hidrológicas relativamente homogéneas, consideradas relevantes para a determinação das condições ecológicas. Em Portugal Continental foram definidos 15 tipos de rios (ver INAG, 2008a). O termo "condições de referência" indica características físico-químicas, hidromorfológicas e biológicas não alteradas e concentrações de poluentes próximas de zero (Bouleau & Pont, 2015). O estado ecológico de referência é, assim, um estado, no presente ou no passado, que corresponde à ausência de pressões antrópicas significativas, isto é, onde os efeitos da industrialização, urbanização ou intensificação da agricultura não sejam visíveis (INAG, 2009). A DQA estabelece, assim, a realização de um Exercício de Intercalibração prévio à implementação da regulamentação, com a finalidade de assegurar a consistência e comparabilidade dos sistemas de monitorização dos vários Estados-Membros. Para isso, os vários Estados-Membros foram organizados em Grupos de Intercalibração Geográficos (GIG), que partilham tipos de massas de água comuns. Portugal integrou o GIG Mediterrâneo – Rios, juntamente com Chipre, Espanha, França, Grécia, Itália e Eslovénia (INAG, 2009).

Com a aprovação da DQA, a monitorização dos ecossistemas aquáticos passou a reger-se por um novo paradigma, que abandona a abordagem clássica da água como recurso (perspetiva antropocêntrica), centrando-se agora na água como suporte de ecossistemas (perspetiva ecocêntrica) (INAG, 2008b). Nesta nova visão da DQA, a definição do estado ecológico da massa da água baseia-se em i) elementos de qualidade biológica (fitobentos, invertebrados bentónicos, macrófitas, fauna piscícola); ii) elementos químicos e físico-químicos, incluindo elementos físico-químicos gerais (p. ex., pH, oxigénio dissolvido, nitratos) e poluentes presentes em quantidades significativas, designados por poluentes específicos (p. ex., bifenilo policlorado, xilenos, propanil, dimetoato, cobre, prata;

anexo B (INAG, 2009)) e iii) elementos hidromorfológicos (regime hidrológico, continuidade do rio e condições morfológicas). De acordo com os resultados da 1ª fase do Exercício de Intercalibração, são utilizados na classificação do estado ecológico em rios os elementos biológicos fitobentos (diatomáceas) e os invertebrados bentónicos, devido à vasta informação existente sobre a autoecologia da maior parte das espécies, em relação à sua tolerância à poluição difusa, de origem orgânica, assim como chaves de identificação completas e índices bióticos utilizados antes da implementação das metodologias da DQA. Outros elementos de qualidade biológica como as macrófitas e a fauna piscícola não serão utilizados na avaliação do estado ecológico, pois apenas serão intercalibrados na 2ª fase do Exercício de Intercalibração e já que os índices desenvolvidos/adotados nos trabalhos de implementação da DQA com estes grupos apresentam um grau de fiabilidade insuficiente (INAG, 2009). A classificação final dos elementos biológicos corresponderá à pior classificação obtida por cada indicador (princípio *one out – all out*; INAG, 2009). O mesmo acontecerá com os restantes elementos caracterizadores do estado ecológico (elementos químicos/físico-químicos e hidromorfológicos). Desta forma, estado ecológico de uma massa de água é determinado pelo elemento de qualidade ecológica que apresente a pior classificação, ou seja, o elemento mais afetado pelas alterações que foram induzidas no sistema em análise. No final, o estado ecológico é expresso nas classes Excelente, Bom, Razoável, Medíocre e Mau (INAG 2009). Para efeitos de comunicação gráfica, a estas classes correspondem, respetivamente, as cores azul, verde, amarelo, laranja e vermelho.

O estado das massas de água de superfície é, também, definido em função do seu estado químico. Este está relacionado com a presença de substâncias químicas no ambiente aquático que, em condições naturais não estariam presentes ou estariam presentes em concentrações reduzidas, e que são suscetíveis de causar danos significativos para a saúde humana e para a flora e fauna, pelas suas características de persistência, toxicidade e bioacumulação. Os elementos de qualidade relevantes para avaliar o estado químico das águas superficiais são as substâncias prioritárias e outros poluentes (INAG 2009). O DL n.º 103/2010 de 24 de Setembro define «substâncias prioritárias» como aquelas que representam risco significativo para o ambiente aquático, sendo a sua identificação feita através de procedimentos de avaliação de risco legalmente previstos ou, por razões de calendário, através de avaliações de risco simplificadas (p. ex., benzeno, mercúrio e compostos de mercúrio – anexo I do DL); define também «outros poluentes» como aqueles que fazem parte do grupo das substâncias que requerem medidas específicas com o objetivo de conseguir o bom estado químico das águas (p. ex., aldrina, DDT total – anexo II do DL). O DL refere, ainda, no anexo III, as normas de qualidade ambiental para as substâncias prioritárias e outros poluentes, ou seja, os níveis máximos de concentração destas substâncias na água, que não devem ser ultrapassados para proteção da saúde humana e do ambiente. O estado químico é determinado pela substância que apresente a pior classificação, sendo apenas expresso nas classes Bom e Insuficiente (cores azul e vermelho, respetivamente), que depois se traduzem nas classes Excelente/ Bom e Razoável/ Medíocre/ Mau para facilitar a comparação com os elementos da qualidade ecológica (INAG 2009).

A figura 2 representa, de forma esquemática e concetual, o sistema de classificação e a forma como os diferentes elementos de qualidade devem ser combinados para classificar o estado ecológico e o estado químico, bem como para obter o estado da massa de água de superfície.

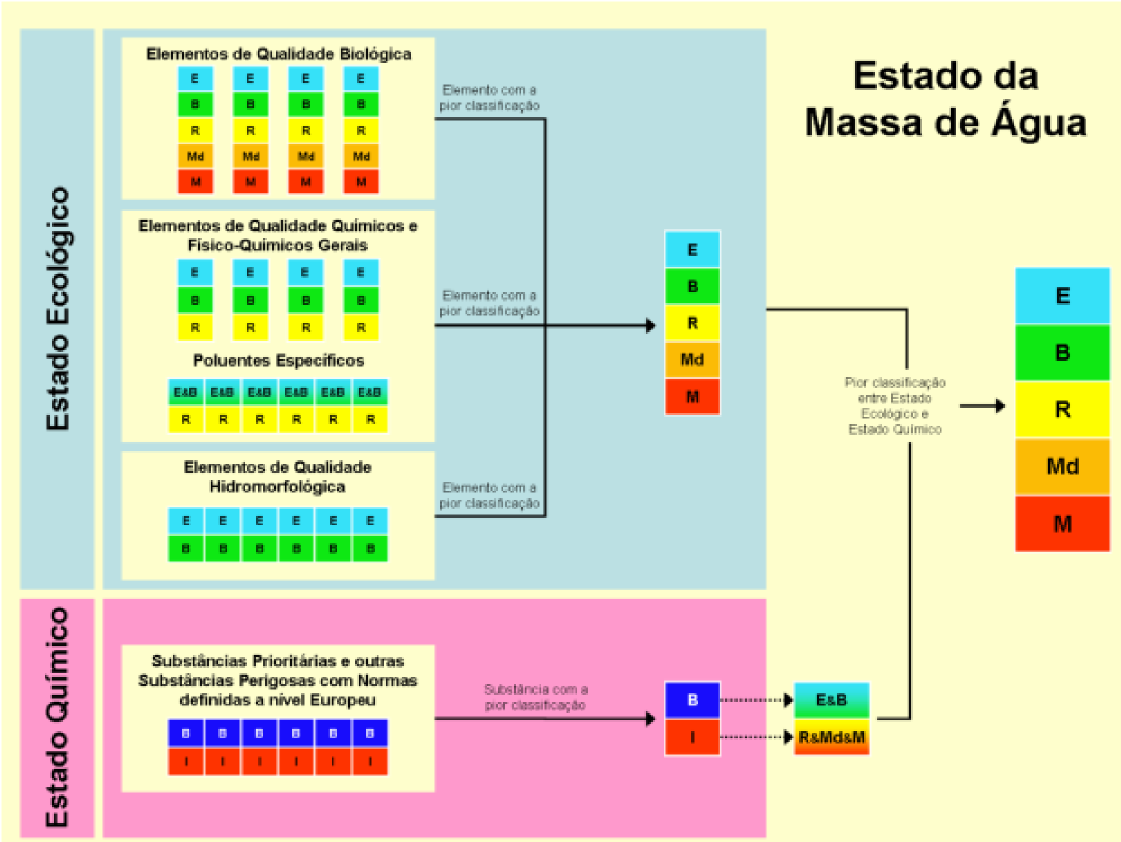


Fig 2. Esquema concetual do sistema de classificação da DQA, onde E – Excelente, B – Bom, R – Razoável, Md – Medíocre, M – Mau, I - Insuficiente (retirado de INAG, 2009).

Embora a DQA seja um instrumento bastante abrangente desenvolvido para proteger os ecossistemas dulçaquícolas na Europa, existem algumas preocupações relativas à sua implementação e vários estudos têm questionado a sua efetividade enquanto ferramenta de regulamentação e proteção ambiental. Apesar dos enormes esforços aplicados no exercício de intercalibração, nem todos os métodos de avaliação foram, ainda, harmonizados. Esta lacuna é, particularmente, importante para as águas de transição, que sofrem de múltiplas pressões, condições de referência indefinidas e falta de dados históricos (Reyjol et al., 2014). Estas limitações aplicam-se, também, a lagos, apesar de haver mais dados sobre estas massas de água, facilitando a definição de condições de referência, e a grandes rios (Birk et al., 2012). Apesar disso, há ausência de dados adequados e comparáveis entre os vários países. Em alguns casos, uma grande quantidade de dados foram recolhidos, mas com diferentes métodos, o que dificulta a identificação de um conjunto de dados adequados para comparação (Poikane et al., 2014).

Outro aspeto desafiante da DQA é a "identificação" de condições de referência, em que a escassez de locais não perturbados é cada vez mais evidente para a maioria das regiões e tipos de massas de águas na Europa (Poikane et al., 2014). Isto deve-se ao contexto atual de mudanças ambientais e às interações contantes e crescentes entre o Homem e a natureza (Bouleau & Pont, 2015).

A monitorização química é também preocupante, na medida em que requer um conhecimento *a priori* do tipo de substâncias que devem ser monitorizadas, uma vez que, por razões técnicas e económicas, não é possível analisar, detetar e quantificar todas as substâncias que estão presentes no ambiente aquático. Além disso, para estimar o risco de efeitos relacionados com o grande número de substâncias que estão presentes no meio seria necessário desenvolver um grande número de critérios de avaliação (Wernersson et al., 2015). Estes critérios são geralmente desenvolvidos substância a substância, com base em estudos de laboratório e, normalmente, não consideram as consequências da exposição simultânea a vários produtos químicos que ocorrem no ambiente, produzindo possivelmente efeitos cumulativos (CE 252, 2012).

Apesar dos aspetos acima referidos, a DQA foi um passo muito importante na avaliação da qualidade da água, que beneficia os países parceiros da UE, uma vez que os rios podem, agora, ser vistos e geridos como ecossistemas, por um quadro legislativo único que estabelece padrões uniformes na política da água, respeitando as diferentes ecorregiões (Moog, Schmidt-Kloiber, Ofenböck, & Gerritsen, 2004) e tipologias dos rios (Verdonschot & Nijboer, 2004). No entanto, em Portugal são escassos os estudos que incidem sobre o estado de qualidade da água dos rios e, na sua maioria, encontram-se sustentados em medições de parâmetros físico-químicos, ignorando a vertente ecológica dos cursos de água. A entrada em vigor da Lei da Água, vem reforçar, assim, a necessidade de avaliar e monitorizar os sistemas aquáticos de forma integrada, através da monitorização de parâmetros hidromorfológicos, físico-químicos e biológicos, contribuindo para o seu bom estado e para uma gestão sustentável dos recursos naturais.

2.1. *Elementos da qualidade biológica*

A importante inovação da DQA é, sem dúvida, a integração de abordagens químicas e ecológicas para monitorizar e gerir o ambiente aquático (Bettinetti, Ponti, Marziali, & Rossaro, 2012). A diretiva requer que a monitorização química de poluentes prioritários, através de procedimentos de química analítica, seja uma prática padrão, mas a nova abordagem enfatiza a importância da monitorização biológica, uma vez que a diversidade de organismos que habita nos ambientes aquáticos reflete a estrutura e o funcionamento geral dos ecossistemas (Gottardo et al., 2011). Neste contexto, embora a DQA atualmente exija apenas a monitorização de fitobentos (diatomáceas) e macroinvertebrados bentónicos (vide secção anterior), os elementos de qualidade biológica relevantes para a avaliação do estado ecológico dos rios a considerar no futuro são também comunidades de macrófitas e a fauna piscícola (INAG, 2009). A tabela 2 evidencia algumas vantagens e desvantagens do uso de diferentes grupos de organismos como indicadores da qualidade da água.

Tabela 2. Vantagens e desvantagens de diferentes grupos de organismos como indicadores da qualidade dos rios (baseado em Hellawell, 1977; Barbour, Gerritsen, Snyder, & Stribling, 1999; Carter, Resh, Hannaford, & Myers, 2006; INAG, 2009).

Organismos	Vantagens	Desvantagens
Macrófitas	Espécies fáceis de encontrar e de identificar. Bons indicadores de sólidos em suspensão e do enriquecimento das águas em nutrientes.	Lacunas de conhecimento quanto à tolerância aos diversos tipos de pressão. Necessária experiência de amostragem e competências na identificação taxonómica. Ocorrência principalmente sazonal.
Fitobentos	Tolerância à poluição orgânica difusa. Colheita fácil e rápida.	Elevadas competências necessárias para a identificação taxonómica.
Macroinvertebrados bentónicos	Amplamente distribuídos e abundantes. Diversidade de formas e hábitos.	Amostragem quantitativa difícil. Conhecimento dos ciclos de vida necessário para

	Resposta da maioria das espécies a diferentes tipos de poluição bem conhecida. Muitas espécies sedentárias podem indicar efeitos de uma perturbação que ocorra no local de amostragem. Toda a comunidade pode responder às alterações ambientais. Amostragem qualitativa fácil. Boas chaves taxonómicas, pelo menos até à família.	interpretar ausência de espécies. Alguns grupos são difíceis de identificar. Variação sazonal pode dificultar comparações ou interpretações dos dados.
Fauna piscícola	Ciclos de vida bem conhecidos bem como a sua distribuição. Bons indicadores dos efeitos de uma perturbação a longo prazo (vários anos). Pode indicar efeitos dos níveis tróficos inferiores.	Lacunas de conhecimento quanto à tolerância aos diversos tipos de pressão. Amostragem morosa e logisticamente complexa. Necessária elevada experiência de amostragem e competências na identificação taxonómica. Espécies podem migrar para evitar poluição.

Quando Hellawell (1986) analisou a literatura, há mais de três décadas, para determinar quais os grupos mais utilizados como indicadores em trabalhos de biomonitorização, reportou os seguintes resultados: macroinvertebrados bentónicos (27% dos estudos); algas (25%); protozoários (17%); bactérias (10%) e peixes (6%); fungos, macrófitas e vírus foram raramente indicados. Mais recentemente, Resh (2008) verificou que os três grupos mais usados na biomonitorização foram os peixes, macroinvertebrados bentónicos e algas.

No presente trabalho, os macroinvertebrados bentónicos foram selecionados, transversalmente, como o grupo bioindicador a integrar em índices bióticos para a avaliação da qualidade ecológica de sistemas lóticos e semi-lóticos. Estando limitada aos dois grupos indicadores que são utilizados a nível nacional (diatomáceas perifíticas e macroinvertebrados bentónicos), esta opção teve em conta o balanço entre as vantagens e desvantagens da utilização de macroinvertebrados (Tabela 1), a sua clara prevalência em estudos de biomonitorização, conforme discutido acima, e ainda a experiência e conhecimento do grupo de trabalho.

2.2. *Índices bióticos*

A metodologia adotada pela DQA, para a avaliação da qualidade ecológica das massas de água com base em elementos biológicos, contempla a utilização de índices bióticos (valores numéricos que expressam o estado geral dos ecossistemas). Estes índices descrevem diferentes aspetos da estrutura e da sensibilidade das comunidades (diversidade, abundância, tolerância) (Martinez-Haro et al., 2015), sendo baseados em dados qualitativos (presença/ ausência) e na identificação taxonómica de alguns grupos de organismos até ao nível da família, no que diz respeito aos macroinvertebrados bentónicos (Leiva, 2004). Foram incluídos em muitos programas de monitorização, uma vez que são fáceis de calcular e podem ser combinados em índices multimétricos numa tentativa de quantificar, com um único valor, o efeito da poluição orgânica nas comunidades, em locais específicos (Bettinetti et al., 2012; Gray & Delaney, 2010).

Os dois índices multimétricos adotados para a classificação das massas de água nacionais são o Índice Português de Invertebrados Norte ($IPtI_N$), aplicado à maioria dos tipos de rios do norte de Portugal e o Índice Português de Invertebrados Sul ($IPtI_S$), definido para a maioria dos rios do sul de Portugal. As métricas que compõem os índices nacionais dos invertebrados bentónicos, bem como os fatores de ponderação de cada métrica e as fórmulas de cálculo, são:

$\mathbf{IPtI_N}$ = Nº *Taxa* x 0,25 + EPT x 0,15 + Evenness x 0,1 + (IASPT – 2) x 0,3 + Log (Sel. ETD+1) x 0,2

$\mathbf{IPtI_S}$ = Nº *Taxa* x 0,4 + EPT x 0,2 + (IASPT – 2) x 0,2 + Log (Sel. EPTCD+1) x 0,2

Onde **EPT** é o número de famílias pertencentes às ordens Ephemeroptera, Plecoptera, Trichoptera; **Evenness**, também designado por índice de Pielou ou Equitabilidade (E = H/ln S, em que H é a diversidade de Shannon-Wiener e S o número de *taxa* presentes); **IASPT** ou ASPT Ibérico, que corresponde ao BMWP Ibérico (Alba-Tercedor & Sanchez-Ortega, 1988) dividido pelo número de famílias incluídas no cálculo do BMWP Ibérico; **Log (Sel. ETD+1)**, Log_{10} de 1 + soma das abundâncias de indivíduos pertencentes às famílias Heptageniidae, Ephemeridae, Brachycentridae, Goeridae, Odontoceridae, Limnephilidae, Polycentropodidae, Athericidae, Dixidae, Dolichopodidae, Empididae, Stratiomyidae e **Log (Sel. EPTCD)**, Log_{10} de 1 + soma das abundâncias de indivíduos pertencentes às famílias Chloroperlidae, Nemouridae, Leuctridae, Leptophlebiidae, Ephemerellidae, Philopotamidae, Limnephilidae, Psychomyiidae, Sericostomatidae, Elmidae, Dryopidae, Athericidae (INAG, 2009).

As métricas utilizadas por estes índices permitem dar resposta às componentes indicadas na DQA, mais propriamente aos elementos biológicos (composição e abundância), permitindo simultaneamente descrever gradientes de degradação geral e discriminar classes de qualidade (INAG, 2009). O cálculo do índice multimétrico é antecedido de uma dupla normalização: cada métrica é dividida pelo correspondente valor de referência, antes de ser multiplicada pelo fator de ponderação; o somatório das métricas normalizadas e ponderadas é dividido pelo respetivo valor de referência, para que o valor final venha expresso em Rácios de Qualidade Ecológica (RQE); os valores de referência de cada tipo de rio derivam dos exercícios de intercalibração e estão disponíveis na Tabela A3 do documento técnico do INAG que descreve os critérios para a classificação do estado de massas de água superficiais (INAG, 2009). Os RQE deverão ser expressos num valor numérico entre 0 (situação de degradação extrema) e 1 (situação de referência).

No entanto, a aplicação dos índices bióticos a outros tipos de poluição ou perturbação (Metcalfe-Smith, 1994) que não a poluição orgânica, tais como metais ou alterações físicas do *habitat* (p. ex., deposição de sedimentos finos, alterações hidrológicas; Extence, Balbi, & Chadd, 1999; Davy-Bowker & Furse, 2006; Dunbar et al., 2010; Extence et al., 2013), pode ser questionável. Um desafio ainda maior da DQA é a discriminação dos efeitos desses diferentes fatores de *stress*, porque eles interagem uns com os outros, obtendo-se um resultado imprevisível em termos de qualidade ecológica (Vidal et al., 2014).

Neste contexto, a análise da estrutura de comunidades com métodos multivariáveis pode ser uma ferramenta importante para complementar a avaliação da qualidade ecológica da água, dada a sua capacidade discriminatória (Vidal et al., 2014). Este tipo de análise é muito usado em Ecologia para estudar variações espaciais e temporais nos ecossistemas. A análise multivariável abrange toda a comunidade, podendo associar a composição da mesma a critérios que caracterizam processos naturais e parâmetros indicadores da presença de poluição (Jeffries & Mills, 1996), ou seja, descreve padrões e relações entre comunidades bióticas e condições ambientais (Hawkins, Norris, Hogue, & Feminella, 2014; Vidal et al., 2014). A principal vantagem deste tipo de análise é a integração de dados multidimensionais com o mínimo possível de perda de informação, permitindo a deteção de tendências de variabilidade pouco evidentes nos dados (Rosemberg & Resh, 1993). Por exemplo, os métodos de ordenação distribuem os dados em análise num sistema de eixos coordenados, de modo a refletir a similaridade das suas relações, ou seja a sua posição relativa em relação aos eixos e entre cada elemento, fornecendo o máximo de informação sobre as semelhanças ecológicas. As relações entre espécies e amostras são representadas num espaço reduzido (duas ou três dimensões), de forma a permitir paralelamente a fácil interpretação dos gradientes ambientais ao longo da estrutura representada. Nos diagramas de ordenação, e de uma forma generalista, pode assumir-se que as amostras que se encontram próximas umas das outras terão fatores ambientais subjacentes similares, ou seja, na ordenação, as espécies com exigências ecológicas semelhantes têm tendência a ficarem próximas no diagrama (Mereta, Boets, Meester, & Goethals, 2013).

Existem várias técnicas de ordenação, entre as quais se podem destacar a análise indireta de gradientes que inclui a análise de correspondências “detrended” (DCA) e a análise direta de gradientes, como a análise de correspondências canónicas (CCA). A DCA é usada para analisar os gradientes na estrutura da comunidade (ocorrência de espécies ou dados de abundância), os quais são interpretados com a ajuda de fontes externas de conhecimento e de dados sobre as variáveis ambientais. Este método é uma técnica de ordenação baseada na média ponderada, sendo mais utilizado em ecologia de comunidades, uma vez que assume um modelo matemático unimodal (Ter Braak, 1995, Gauch, 1982). A CCA é uma extensão da média ponderada que permite avaliar o grau de associação entre as comunidades bióticas e as variáveis ambientais conhecidas (Ter Braak, 1986). Desta forma, a análise multivariável permite um estudo mais detalhado e holístico das comunidades e uma identificação da causa dos impactos observados. Tal potencial tem sido explorado em estudos focados nas comunidades de macroinvertebrados (p. ex., Walsh, 1997; Lücke & Johnson, 2009; Vidal et al., 2014).

3. Sociedade Interventiva

Nas últimas décadas, a comunidade científica e as agências reguladoras tornaram-se cada vez mais conscientes dos impactos, a longo prazo, do aumento da população humana e da sua influência no clima, na sustentabilidade dos ecossistemas e na biodiversidade. Estas preocupações aumentaram, devido aos sucessivos relatórios sobre a destruição da camada de ozono, as alterações climáticas globais, a contaminação do solo e dos recursos hídricos e a extinção de espécies.

Relativamente à biodiversidade, a sua manutenção é importante por várias razões, incluindo a formação de solos, a produção de alimentos, a purificação da água, a degradação ou decomposição de resíduos, a manutenção da composição de gases na atmosfera e outras funções importantes que contribuem para a estabilidade fundamental dos ecossistemas (Spray & McGlothlin, 2003). Recentemente, a problemática da perda de biodiversidade ganhou uma atenção especial (Downes, 1995; Spray & McGlothlin, 2003). A Convenção sobre a Diversidade Biológica (CDB) foi um dos acordos internacionais, sobre o desenvolvimento sustentável, aprovada durante a Conferência das Nações Unidas para o Ambiente e Desenvolvimento (Cimeira da Terra), que decorreu no Rio de Janeiro em junho de 1992. Nesta Convenção, a biodiversidade foi definida como a variabilidade entre os organismos vivos de todas as origens, incluindo os ecossistemas aquáticos terrestres, marinhos e outros ecossistemas aquáticos, e os complexos ecológicos de que fazem parte; isto inclui a diversidade dentro de espécies, entre espécies e dos ecossistemas (CDB, 1992). Assim, o conceito de biodiversidade está associado à variação genética dentro das espécies, à diversidade de espécies dentro dos ecossistemas (riqueza de espécies) e à diversidade dos ecossistemas. Todas estas dimensões estão a ser ameaçadas pelas atividades humanas (Downes, 1995; Spray & McGlothlin, 2003). A perturbação do equilíbrio de qualquer uma dessas dimensões tem uma relação direta com o declínio da diversidade e consequentemente há um aumento da vulnerabilidade do sistema às pressões ambientais e da probabilidade de extinção de espécies.

Em maio de 2011, a Comissão Europeia adotou uma nova estratégia que estabelece o quadro de ação da UE para os próximos dez anos com vista a atingir o objetivo central para 2020 em matéria de biodiversidade que foi fixado pelos seus dirigentes em março de 2010 e pelos 193 países que participaram, incluindo a UE e todos os seus Estados-Membros, na Conferência das Partes à Convenção sobre Diversidade Biológica, realizada em Nagoia, no Japão, em 2010 (CE, 2011). Tanto a CDB como a Conferência das Partes confirmaram a necessidade de sensibilizar e educar a sociedade para a compreensão do valor da biodiversidade e de provocar a mudança através do desenvolvimento de programas de educação e sensibilização do público (Jiménez, Díaz, Monroe, & Benayas, 2014). A importância do envolvimento do cidadão na compreensão e monitorização da biodiversidade, bem como a sua participação no desenvolvimento de políticas ambientais eficazes, foi claramente expresso pela Comissão Europeia, no documento "Horizon 2020" (CE, 2014).

A exposição de informação científica pode ajudar a envolver os cidadãos em campanhas de ciência, como por exemplo, campanhas que visem a monitorização das mudanças na distribuição de espécies e composição a curto, médio e longo prazo (Martellos & Nimis, 2015). Biólogos e outros profissionais podem aumentar, assim, a sua capacidade de compreensão dos desafios a nível local e gerir os ecossistemas locais se trabalharem juntamente com a sociedade local (Nerbonne & Nelson, 2008). Desta forma, é fomentada a aprendizagem social, em que todos os cidadãos compartilham diversas perspetivas e experiências no sentido de desenvolverem um quadro comum de informação e uma base para uma ação conjunta (Schusler, Decker, & Pfeffer, 2013). Assim, com o planeamento de iniciativas de conservação, através de parcerias, os profissionais e outros cidadãos podem influenciar, efetivamente, a política ambiental (Norton, 2003). Neste contexto, para se criar uma "ponte" entre profissionais e comunidade, será necessário criar ferramentas ambientais visualmente atrativas e fáceis de compreender e interpretar.

A identificação de macroinvertebrados bentónicos, através da utilização de chaves dicotómicas, pode ser uma das ferramentas utilizadas para estudar a qualidade ecológica de ecossistemas lóticos. No entanto, a utilização destas ferramentas pode ser difícil para muitas pessoas com pouca informação sobre estes organismos. Estas chaves são constituídas por sequências de parágrafos alternativos para uma ou para um nº reduzido de características da morfologia externa dos macroinvertebrados bentónicos. As fotografias/esquemas que ilustram as estruturas descritas ajudam o utilizador a interpretar as descrições constantes de cada passo da chave (Randler, 2008). Este tipo de recurso já não se limita apenas a um público académico, mas pode ser também relevante para leigos, como amadores, turistas, que podem ter acesso, e, eventualmente, compartilhar novos conhecimentos (Martellos & Nimis, 2015). Por isso, as chaves de identificação devem ser construídas de forma a satisfazer as necessidades do utilizador de forma simples e eficiente (Martellos & Nimis, 2015). No entanto, as chaves para os "não-especialistas" não devem ser demasiadamente simplistas, para não diminuir a sua fiabilidade (Stagg, Donkin, & Smith, 2014).

A criação de chaves dicotómicas ilustradas pode ser, assim, uma forma de colocar ao alcance de todos, ou quase todos, a aquisição do conhecimento, divulgação e preservação dos ecossistemas dulçaquícolas.

4. Objetivos e Estrutura da Dissertação

O presente trabalho teve como objetivo global avaliar a qualidade ecológica de sistemas lóticos selecionados, tendo em conta a forma como diferentes agentes de *stress* (físicos, químicos e biológicos) identificados nesses sistemas afetam a estrutura das comunidades de macroinvertebrados bentónicos. Utilizando abordagens de análise abrangentes e diversas, em paralelo à linha metodológica e de análise de dados recomendada no âmbito da avaliação do estado ecológico de sistemas lóticos pela DQA, pretende-se discutir se estas últimas oferecem a resolução necessária para distinguir impactos de agentes de *stress* que não a poluição difusa, para a qual a DQA terá sido, no geral, definida. Assim, os objetivos específicos deste trabalho foram:

✍ avaliar a qualidade ecológica do rio Sabor e de alguns dos seus tributários, analisando possíveis impactos associados à construção de uma barragem (agente de *stress* físico);

✍ avaliar a qualidade ecológica de alguns cursos de água envolventes à zona mineira de S. Domingos, estudando os possíveis efeitos da drenagem ácida (agente de *stress* químico);

✍ avaliar a qualidade ecológica da rede de canais de Mira, tendo em conta possíveis implicações da espécie invasora *Corbicula fluminea* (agente de *stress* biológico);

✍ desenvolver e validar uma chave dicotómica, fotográfica, para a identificação de espécies de macroinvertebrados existentes em Portugal, de forma a envolver a sociedade na manutenção e proteção de ecossistemas dulçaquícolas.

Abordando cada um dos objetivos específicos acima mencionados em particular, a presente Tese está dividida em seis capítulos que registam os estudos efetuados.

No capítulo I (presente capítulo) é feita uma revisão do estado-da-arte, no que diz respeito aos impactos das ações antrópicas nos sistemas lóticos e os critérios estabelecidos pela DQA para a avaliação ambiental e melhoria do estado ecológico das massas de água. Destaca-se, também, a importância da comunidade de macroinvertebrados por ser um bom indicador da condição e "estado de saúde" do sistema lótico. Desta forma, estabelece-se a introdução geral ao trabalho realizado, seguida de quatro capítulos individualizados, que constituirão manuscritos científicos a publicar em revistas científicas ou livros de circulação nacional/internacional, contribuindo todos para uma abordagem consistente e integrada à problemática focada nesta Tese. No capítulo II são analisados os possíveis efeitos na comunidade de macroinvertebrados bentónicos, provocados pela construção de uma barragem, no troço inferior do rio Sabor. No capítulo III são analisados os possíveis efeitos da drenagem ácida proveniente da mina de S. Domingos nas comunidades de macroinvertebrados bentónicos. No capítulo IV são discutidas as possíveis implicações da espécie invasora *Corbicula fluminea* na qualidade ecológica da rede de canais de Mira e, concomitantemente, são analisadas as preferências ecológicas da espécie, o que constitui informação crítica para o desenvolvimento de estratégias de gestão da qualidade ecológica dos ecossistemas invadidos. No capítulo V apresenta-se o desenvolvimento e validação de uma chave de identificação, fotográfica, de macroinvertebrados bentónicos existentes em Portugal. Este capítulo representa a integração da problemática num contexto de envolvimento da sociedade nas questões ambientais prementes da atualidade. Por último, no capítulo VI, efetua-se uma discussão geral de todos os resultados obtidos, apresentando-se o conjunto de conclusões mais relevantes que puderam ser extraídas dos diferentes estudos efetuados.

A figura 3 apresenta esquematicamente a Tese, evidenciando a integração entre os diferentes capítulos e a lógica das opções tomadas para a seleção dos sistemas estudados e agentes de *stress* abordados.

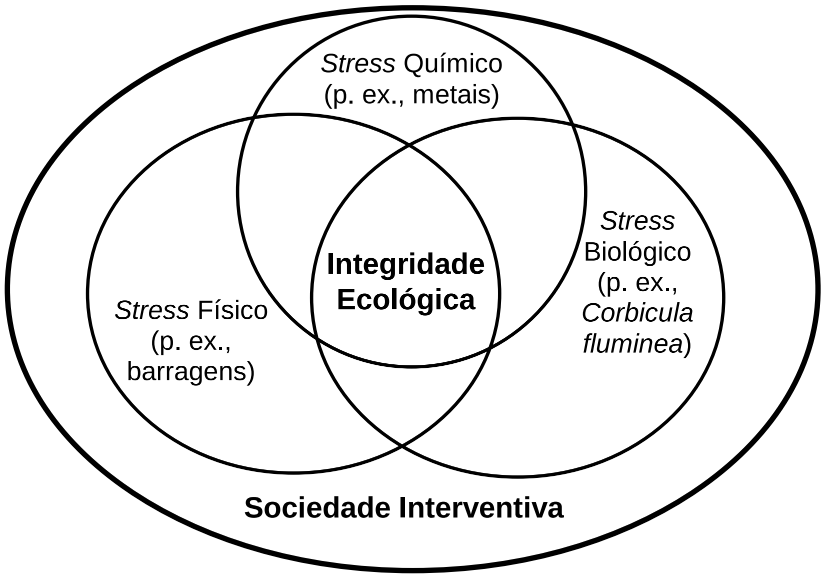


Fig. 3. Esquema global da Tese.

5. Referências Bibliográficas

Agência Portuguesa do Ambiente (2016). Barragens de Portugal. Disponível em http://www.apambiente.pt/index.php?ref=77&subref=839.

Akcil, A., & Koldas, S. (2006). Acid mine drainage (AMD): causes, treatment and case studies. *Journal of Cleaner Production*, 14, 1139–1145.

Alba-Tercedor, J., & Sánchez-Ortega, A. (1988). Um método rápido y simple para evaluar la calidad de las águas corrientes basado em el de Hellawell (1978). *Limnetica*, 4, 51-56.

Aldridge, D.W., & McMahon, R.F. (1978) Growth, fecundity, and bioenergetics in a natural population of the Asiatic freshwater clam, *Corbicula manilensis* Philippi, from north central Texas. *Journal of Mollus*, 44, 49–70.

Araújo, R., Moreno, D., & Ramos, M.A. (1993) The Asiatic clam *Corbicula fluminea* (Müller, 1774) (Bivalvia: Corbiculidae) in Europe. *American Malacological Bulletin*, 10, 39–49.

Barbour, M.T., Gerr itsen, J., Snyder, B.D., & Stribling, J.B. (1999). *Rapid Bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish*. Washington: Environmental Protection Agency; Office of Water.

Battaglia, M., Hose, G.C., Turak, E., & Warden, B. (2005). Depauperate macroinvertebrates in a mine affected stream: clean water may be key to recovery. *Environmental Pollution*, 138, 132–141.

Bettinetti, R., Ponti, B., Marziali, L., & Rossaro, B. (2012). Biomonitoring of lake sediments using benthic macroinvertebrates. *Trends in Analytical Chemistry*, 36, 92-102.

Biggs, B. (1985). Algae, a blooming nuisance in rivers. *Soil & Water*, 21, 21-31.

Biggs, B.J.F., & Price, G.M. (1987). A filamentous algal proliferations survey of in New Zealand Rivers. *Journal of Marine Freshwater Research*, 21, 175-191.

Biggs, B.J. (2000). Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae. *Journal of North American Benthological Society*, 19(1), 17-31.

Birk, S., Bonne, W., Borja, A., Brucet, S., Courrat, A., Poikane, S., … Hering, D. (2012). Three hundred ways to assess Europe's surface waters: an almost complete overview of biological methods to implement the Water Framework Directive. *Ecological Indicators*, 18, 31–41.

Britton, J.C. (1982) Biogeography and Ecology of the Asiatic clam, Corbicula, in Texas. *Proceedings of the Symposium on Recent Benthological Investigations in Texas and adjacent States* (ed J.R. Davis), pp. 21–31. Aquatic Sciences Section, Texas Academy of Science, Austin, Texas.

Bouleau, G., & Pont, D. (2015). Did You Say Reference Conditions? Ecological and Socio-economic Perspectives on the European Water Framework Directive. *Environmental Science and Policy*, 47, 32-41.

Camargo, J. A., Alonso, A., & Puente, M. (2005). Eutrophication downstream from small reservoirs in mountain rivers of central Spain. *Water Research*, 39, 3376–3384.

Cantanhede, G., Hahn, N.S., Gubiani, E.A., & Fugi, R. (2008). Invasive molluscs in the diet of *Pterodoras granulosus* (Valenciennes, 1821) (Pisces, Doradidae) in the Upper Parana River floodplain, Brazil. *Ecology of Freshwater Fish*, 17, 47-53.

Carter, J.L., Resh, V.H., Hannaford, M.J., & Myers, M.J. (2006). Macroinvertebrates as Biotic Indicators of Environmental Quality. In F.R. Hauer & G. Lamberti, *Methods in Stream Ecology* (2nd ed., pp. 806-833). London: Academic press.

CE (2000). Diretiva 2000/60/CE de 23 de Outubro. Jornal Oficial das Comunidades Europeias, L327. Parlamento Europeu e do Conselho, Portugal.

CE (2010). *Diretiva Quadro Água*. Luxemburgo: Serviço das Publicações da União Europeia.

CE (2014). Horizon 2020. Luxemburgo: Serviço das Publicações da União Europeia.

CE (2011). *Estratégia de biodiversidade da UE para 2020*. Luxemburgo: Serviço das Publicações da União Europeia.

CE 670 (2012). *Relatório da comissão ao parlamento europeu e ao conselho sobre a execução da Diretiva-Quadro Água (2000/60/CE). Planos de gestão de bacia hidrográfica*. Bruxelas: Comissão Europeia.

CE 252 (2012). *Comunicação da comissão ao conselho. Efeitos da combinação de produtos químicos*. Bruxelas: Comissão Europeia.

Céréghino, R., Cugny, P., & Lavandier, P. (2002). Influence of intermittent hydropeaking on the longitudinal zonation patterns of benthic invertebrates in a mountain stream. *International Review of Hydrobiology*, 87, 47–60.

Christopher, A.T., Granata, T.C., Murphy, R.P., Livchak, C.J. (2007). Using a coupled eco-hydrodynamic model to predict habitat for target species following dam removal. *Ecological Engineering*, 30, 215–230.

Chung, P.R., Jung, Y., Park, Y.K., Hwang, M.G., & Soh, C.T. (2001). *Corbicula fluminea* (Bivalvia: Corbiculidae): A possiblesecond molluscan intermediate host of *Echinostoma cinetorchis* (Trematoda: Echinostomatidae) in Korea. *Korean Journal of Parasitology*, 39, 329-332.

Cohen, R.R.H., Dresler, P.V., Phillips, E.J.P., & Cory, R.L. (1984). The effect of the asiatic clam, *Corbicula fluminea*, on phytoplankton of the Potomac River, Maryland. *Limnology and Oceanography*, 29, 170-180.

Connell, D. (1999). *Introduction to Ecotoxicology*. Oxford: Blackwell Science.

Connell, D.W. (2005). *Basic concepts of environmental chemistry*, 2nd ed.. New York: Taylor & Francis Group, LLC.

Crompton, T.R. (1997). *Toxicants in the Aqueous Ecosystem*. England: Jonh Wiley & Sons.

DAISIE – European Invasive Alien Species Gateway (2006). *Dreissena polymorpha*. Retrieved from http:// www. europe – aliens. org/ speciesFactsheet. do? speciesId= 50169.

DAISIE – European Invasive Alien Species Gateway (2008). *Corbicula fluminea*. Retrieved from http:// www. europe – aliens. org/ speciesFactsheet. do? speciesId= 53281.

Darrigran, G. (2002) Potential impact of filter-feeding invaders on temperate inland freshwater environments. *Biological Invasions*, 4, 145–156.

Davy–Bowker, J., & Furse, M.T. (2006). Hydromorphology – major results and conclusions from the STAR project. *Hydrobiologia*, 566, 263–265.

DeNicola, D.M., & Stapleton, M.G. (2002). Impact of acid mine drainage on benthic communities in streams: the relative roles of substratum vs. aqueous effects. *Environmental Pollution*, 119, 303-315.

Downes, D.R. (1995). Global trade, local economies and the Biodiversity Convention. In W.J. Snape (Ed.), *Biodiversity and the Law* (pp. 202-205). Washington: Island Press.

Dunbar, M.J., Warren, M., Extence, C., Baker, L., Cadman, D., Mould, D.J., … Chadd, R. (2010). Interaction between macroinvertebrates, discharge and physical habitat in upland rivers. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 20, S31–S44.

EDP (2016). Novas barragens. Disponível em http:// www.a-nossa-energia.edp.pt /centros_produtores/empreendimento_type.php?e_type=nb.

El Khalil, H., El Hamiani, O., Bitton, G., Ouazzani, N., & Boularbah, A. (2008). Heavy metal contamination from mining sites in South Morocco: Monitoring metal content and toxicity of soil runoff and groundwater. *Environmental Monitoring and Assessment*, 136,147–160.

Extence, C.A., Balbi, D.M., & Chadd, R.P. (1999). River flow indexing using British benthic macroinvertebrates: a framework for setting hydroecological objectives. *Regulated Rivers: Research and Management*, 15, 545–574.

Extence, C.A., Chadd, R.P., England, J., Dunbar, M.J., Wood, P.J., & Taylor, E.D. (2013). The assement of fine sediment accumulation in rivers using macro-invertebrate community response. *River Research and Applications*, 29, 17–55. *Environmental Pollution*, 130, 263-274.

Gallardo, B, Clavero, M., Sanchez, M.I., Vilà, M. (2016). Global ecological impacts of invasive species in aquatic ecosytems. *Global Change Biology*, 22, 151-163.

Gangloff, M.M. (2011). Taxonomic and ecological tradeoffs associated with small dam removals. *Aquatic conservation: marine and freshwater ecosystems*. 23, 475-480.

Garcia, R. (2015). Metade dos cursos de água em Portugal falha meta europeia de qualidade. Disponível em http://www.publico.pt/ecosfera/noticia/metade-dos-curso-de-agua-em-portugal-ainda-estao-poluidos-1705672.

Gauch, H.G. (1982). *Multivariate analysis in community ecology*. New York: Cambridge University Press.

Gerhardt, A., Bisthoven, L.J., & Soares, A.M.V.M. (2004). Macroinvertebrate response to acid mine drainage: community metrics and on-line behavioural toxicity bioassay. *Environmental Pollution*, 130, 263-274.

Ghate, S., & Chaphekar, S.B. (2000). *Plagiochasma appendiculatum* as a biotest for water quality assessment. *Environmental Pollution*, 108, 173-181.

Giller, P.S., & Malmqvist, B. (1998). *The Biology of Streams and Rivers*. Oxford University Press: New York, USA.

Giller, P.S., & Malmqvist, B. (2000). *The Biology of Streams and Rivers*. Great Britain: Oxford University Press.

Gomes, M.E.P., Antunes, I.M.H.R., Silva, P.B., Neiva, A.M.R., & Pacheco, F.A.L. (2010). Geochemistry of waters associated with the old mine workings at Fonte Santa (NE of Portugal). *Journal of Geochemical Exploration*, 105, 153-165.

Gottardo, S., Semenzin, E., Giove, S., Zabeo, A., Critto, A., Zwart, D., … Ginebreda, A. (2011). Integrated Risk Assessment for WFD Ecological Status classification applied to Llobregat river basin (Spain). Part II — Evaluation process applied to five environmental Lines of Evidence. *Science of the Total Environment*, 409, 4681-4692.

Gray, N.F. (1996). A substrate classification index for the visual assessment of the impact of acid mine drainage in lotic systems. *Water Research*, 30, 1551–1554.

Gray, N.F. (1997). Environmental impact and remediation of acid mine drainage: a management problem. *Environmental Geology*, 27, 358-361.

Gray, N.F. (1998). Acid mine drainage composition and the implications for its impact on lotic systems. *Water Research*, 32, 2122–2134.

Gray, N.F., & Delaney, E. (2010). Measuring community response of bentic macroinvertebrates in an erosional river impacted by acid mine drainage by use of a simple model. *Ecological Indicators*, 10, 668-675.

Grill, G., Dallaire, C.O., Chouinard, E.F., Sindorf, N., & Lehner, B. (2014). Development of new indicators to evaluate river fragmentation and flow regulation at large scales: A case study for the Mekong River Basin. *Ecological Indicators*, 45: 148-159.

Hakenkamp, C.C., & Palmer, M.A. (1999). Introduced bivalves in freshwater ecosystems: the impact of Corbicula on organic matter dynamics in a sandy stream. *Oecologia*, 119, 445-451.

Hakenkamp, C.C., Ribblett, S.G., Palmer, M.A., Swan, C.M., Reid, J.W., & Goodison, M.R. (2001). The impact of an introduced bivalve (*Corbicula fluminea*) on the benthos of a sandy stream. *Freshwater Biology*, 46, 491-501.

Harding, J.S., Quinn, J.M., & Hickey, C.W. (2000). Effects of mining and forestry production: 230-259. In: K.J. Collier & M.J. Winterbourn (Eds). *New Zealand stream invertebrates: ecology and implications for management* (pp. 230-259). Christchurch: New Zealand Limnological Society.

Hawkins, C.P., Norris, R.H., Hogue, J.N., & Feminella, J.W. (2000). Development and evaluation of predictive models for measuring the biological integrity streams. *Ecological Applications*, 10, 1456–1477.

Hellawell, J.M. (1977). Biological surveillance and water quality monitoring. In J.S. Alabaster (Ed.), *Biological Monitoring of Inland Fisheries* (pp. 69-88). London: Applied Science Publishers Ltd.

Hellawell, J.M. (1986). *Biological indicators of freshwater pollution and environmental management*. USA: Elsevier Applied Science.

Hickey, C.W., & Golding, L.A. (2002). Response of macroinvertebrates to copper and zinc in a stream mesocosm. *Environmental Toxicology and Chemistry*, 21, 1854-1863.

Hoyer, A.B., Schladow, S.G., & Rueda, F.J. (2015) Local dispersion of nonmotile invasive bivalve species by wind-driven lake currents. *Limnology and Oceanography*, n/a–n/a.

Hudson-Edwards, K.A., Jamieson, H.E., & Lottermoser, B.G. (2011). Mine wastes: Past, present, future. *Elements*, 7, 375–379.

Hyytiainen, K., Lehtiniemi, M., Niemi, J.K., & Tikka, K. (2013). An optimization framework for adressing aquatic invasive species. *Ecological Economics*, 91, 69-79.

Illari, M.I., Freitas, F., Costa-dias, S., Antunes, C., Guilhermino, L., & Sousa, R. (2012). Associated macrozoobenthos with the invasive Asian clam *Corbicula fluminea*. *Journal of Sea Research*, 72, 113-120.

Illari, M.I., Souza, A.T., Antunes, C., Guilhermino, L., & Sousa, R. (2014). Influence of the invasive Asian clam *Corbicula fluminea* (Bivalvia: Corbiculidae) on estuarine epibenthic assemblages. *Estuarine, Coastal and Shelf Science*, 143, 12-19.

INAG, I.P. (2008a). *Tipologia de rios em Portugal Continental no âmbito da implementação da Directiva Quadro da Água I - caracterização abiótica*. Ministério do Ambiente, Ordenamento do Território e do Desenvolvimento Regional.

INAG, I.P. (2008b). *Manual para a avaliação biológica da qualidade da água em sistemas fluviais segundo a Directiva Quadro da Água - Protocolo de amostragem e análise para os macroinvertebrados bentónicos*. Ministério do Ambiente, Ordenamento do Território e do Desenvolvimento Regional.

INAG, I.P. (2009). *Critérios para a classificação das massas de água superficiais – rios e albufeiras*. Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional.

Jeffries, M., & Mills, D. (1996). *Freshwater Ecology: principles and applications*. Chichester: John Wiley.

Jiménez, A., Díaz, M.J., Monroe, M.C., & Benayas, J. (2014). Analysis of the variety of education and outreach interventions in biodiversity conservation projects in Spain. *Journal for Nature Conservation*, http://dx.doi.org/10.1016/j.jnc.2014.07.002.

Kairo, K., Mols, T., Timm, H., Virro, T., & Jarvekulg, R. (2011).Tthe effect of damming on biological quality according to macroinvertebrates in some estonian streams, central - Baltic Europe: a pilot study. *River Research and Applications*, 27, 895-907.

Kiffney, P.M., & Clements, W.H. (1996). Effects of metals on stream macroinvertebrate assemblages from different altitudes. *Ecological Applications*, 6, 472–481.

Kornijów, R. (2009). Controversies around dams reservoirs: benefits, costs and future. *Ecohydrology & Hydrobiology*, 9(2-4), 141-148.

Kraemer, L.R. & Galloway, M.L. (1986) Larval development of *Corbicula fluminea* (Muller) (Bivalvia, Corbiculacea) - An appraisal of its heterochrony. *American Malacological Bulletin*, 4, 61–79.

Lauritsen, D.D. (1986). Assimilation of radiolabeled algae by Corbicula. *In*: JC Britton (ed.), American Malacological Bulletin - Special edition no.2, Proceedings of the Second International Corbicula Symposium.

Leaf, S.S.,& Chatterjee, R. (1999). Developing a strategy on eutrophication. *Wat. Sci. Tech,* 39(12), 307-314.

Leiva, J. (2004). *Macroinvertebrados bentónicos como bioindicadores de calidad de agua en la Cuenca del estero Peu Peu, Comuna de Lautaro IX, Region de la Araucania* (Tesis de Licenciatura en Recursos Naturales). Universidad Católica de Temuco, Temuco.

Looy, K.V., Tormos, T., & Souchon, Y. (2014). Disentangling dam impacts in river networks. *Ecological Indicators*, 37, 10-20.

Lopes, I., Gonçalves, F., Soares, A.M.V.M., & Ribeiro, R. (1999). Discriminating the ecotoxicity due to metals and to low pH in acid mine drainage. *Ecotoxicology Environmental Safety*, 44, 207–214.

Lücke, J.D., Johnson, R.K. (2009). Detection of ecological change in stream macroinvertebrate assemblages using single metric, multimetric or multivariate approaches. *Ecological Indicators*, 9, 659-669.

Malmqvist, B. & Hoffsten, P.O. (1999). Influence of drainage from old mine deposits on benthic macroinvertebrate communities in central Swedish streams. *Water Research*, 33, 2415-2423.

Malmqvist, B., & Rundle, S. (2002). Threats to the running water ecosystems of the world. *Environmental Conservation*, 29, 134–153.

Marcé, R, Moreno-Ostos, E., García-Barcina, J.Mª., & Armengol, J. (2010). Tailoring dam structures to water quality predictions in new reservoir projects: Assisting decision-making using numerical modelling. *Journal of Environmental Management*, 91, 1255-1267.

Marchant, R., & Hehir, G. (2002). The use of AUSRIVAS predictive models to assess the response of lotic macroinvertebrates to dams in south-east Australia. *Freshwater Biology*, 47, 1033–1050.

Martellos, S., & Nimis, P.L. (2015) From Local Checklists to Online Identification Portals: A Case Study on Vascular Plants. *PLoS ONE*, 10, 1-18.

Martinez-Haro, M., Beiras, R., Bellas, J., Capela, R., Coelho, J.P., Lopes, I., … Marques, J.C. (2015). A review on the ecological quality status assessment in aquatic system using community based indicators and ecotoxicological tools: what might be the added value of their combination. *Ecological Indicators*. 48, 8-16.

McMahon, R.F. (2002) Evolutionary and physiological adaptations of aquatic invasive animals: selection versus resistance. *Canadian Journal of Fisheries and Aquatic Sciences*, 59, 1235–1244.

McCartney, M. (2009). Living with dams: managing the environmental impacts. *Water Policy*, 11, 121-139.

Mereta, S.T., Boets, P., Meester, L., & Goethals, P.L.M. (2013). Development of a multimetric index based on benthic macroinvertebrates for the assessment of natural wetlands in Southwest Ethiopia. *Ecological Indicators*, 29, 510-521.

Metcalfe-Smith, J.L. (1994). Biological Water-Quality Assessment of Rivers: Use of Macroinvertebrate Communities. *In*: Calow, P. & Petts, G. E. (eds.) *The Rivers Handbook*. Oxford: Blackwell Science Ltd.

MINEO (2003). Assessing and monitoring the environmental impact of mining activities in Europe using advanced earth observation techniques. Final Report – Section 6: Detailed Report.

Moog, O., Schmidt-Kloiber, A., Ofenböck, T., & Gerritsen, J. (2004). Does the ecoregion approach support the typological demands of the EU ‘Water Framework Directive’?. *Hydrobiologia*, 516(1), 21-33.

Mouthon, J. (1981). Sur la présence en France et au Portugal de Corbicula (Bivalvia, Corbiculidae) originaire d’ Asie. *Basteria*, 45, 109–116.

Mouthon, J. (2003) Longitudinal and temporal variations of density and size structure of *Corbicula fluminea* (Bivalvia) populations in the Saône and

Rhône rivers (France). *Annales de Limnologie - International Journal of Limnology*, 39, 15–25.

Nebel, B.J., & Wright R.T. (1996). *Environmental science. The way the world works*, 5th ed.. New Jersey: Prentice-Hall International.

Nerbonne, J.F., & Nelson, K.C. (2008). Volunteer macroinvertebrate monitoring: Tensions among group goals, data quality, and outcomes. *Environmental Management*, 42, 470-479.

Nijboer, R.C., & Verdonschot, P.F.M. (2004). Variable selection for modelling effects of eutrophication on stream and river ecosystems. *Ecological Modelling*, 177, 17-39.

Nilsson, C., Reidy, C.A., Dynesius, M., & Revenga, C., (2005). Fragmentation and flow regulation of the world's large river systems. *Science*, 308, 405–408.

Norton, B. (2003). *Searching for sustainability: interdisciplinary essays in the philosophy of conservation biology*. Cambridge, UK: Cambridge University Press.

Oberholster, P.J., Genthe, B., Hobbs, P., Cheng, P.H., Klerk, A.R., & Botha, A.M. (2013). An ecotoxicological screening tool to prioritise acid mine drainage impacted streams for future restoration. *Environmental Pollution*, 176, 244-253.

Odum, E.P. (2004). *Fundamentos de Ecologi*a, 7ª ed.. Lisboa: Fundação Calouste Gulbenkian.

Oliveira, J.M.S., Farinha, J., Matos, J.X., Ávila, P., Rosa, C., Machado, M.J.C., … Leite, M.R.M. (2002). Diagnóstico Ambiental das Principais Áreas Mineiras Degradadas do País. *Boletim de Minas - Instituto Geológico e Mineiro (IGM)*, 39, 67-85.

Palaniappan, M., Gleick, P.H., Allen, L., Cohen, M.J., Christian-Smith, J., Smith, C., & Ross, N. (2010). *Clearing the waters – a focus on water quality solutions*. Kenya: United Nations Environmental Programme.

Phelps, H.L. (1994). The Asiatic clam (*Corbicula fluminea*) invasion and system-level ecological change in the Potomac River estuary near Washington, DC. *Estuaries*, 17, 614-621.

Pimentel, D., Zuniga, R., & Morrison, D. (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics*, 52, 273–288.

Poikane, S., Zampoukas, N., Borja, A., Davies, S.P., van de Bund, W., & Birk, S. (2014). Intercalibration of aquatic ecological assessment methods in the European Union: Lessons learned and way forward. *Environmental Science & Policy*, 44, 237-246.

Prezant, R.S., & Chalermwat, K. (1984). Flotation of the bivalve *Corbicula fluminea* as a means of dispersal. *Science*, 225, 1491-1493.

Quinn, J.M., & Gilliland, B.W. (1989). The Manawatu River cleanup- has it worked? *Transactions of the Institution of Professional Engineers, New Zealand*, 16, 22-26.

Quinn, J.M., & Hickey, C.W. (1990). Magnitude of effects of substrate particle size, recent flooding and catchment development on benthic invertebrate communities in 88 New Zealand rivers. *Journal of Marine and Freshwater Research*, 24, 411-427.

Randler, C., & Birtle, A. (2008). The ‘System of Pasta’–An Introduction to Dichotomous Keys. *School Science Review*, 90, 1–6.

Resh, V.H (2008). Which group is best? Attributes of diferente biological assemblages used in freshwater biomonitoring programs. *Environ. Monit. Assess*, 138, 131-138

Reyjol, Y., Argillier, C., Bonne, W., Borja, A., Buijse, A.D., Cardoso, A.C., … Bund, W. (2014). Assessing the ecological status in the context of the European Water Framework Directive: Where do we go now?, *Science of the Total Environmental*, 497-498, 332-344.

Roline, R.A. (1988). The effects of heavy metals pollution of the upper Arkansas River on the distribution of aquatic macroinvertebrates. *Hydrobiologia*, 160, 3–8.

Rosa, I.C, Costa, R., Gonçalves, F., & Pereira, J. (2011). *Corbicula fluminea*: Utilização de uma espécie invasora como organismo experimental. *CAPTAR*, 1(3), 40-59.

Rosenberg, D.M. & Resh, V.H. (1993). *Freshwater Biomonitoring Macroinvertebrates*. New York: Chapman & Hall.

Santos, E.S., Abreu, M.M., Nabais, C., & Magalhães, M.C.F. (2012). Trace element distribution in soils developed on gossan mine wastes and *Cistus ladanifer* L. tolerance and bioaccumulation. *Journal of Geochemical Exploration*, 123, 45-51.

Schmidlin, S., & Baur, B. (2007). Distribution and substrate preference of the invasive clam *Corbicula fluminea* in the river Rhine in the region of Basel (Switzerland, Germany, France). *Aquatic Sciences*, 69, 153-161.

Schusler, T., Decker, D., & Pfeffer, M. (2003). Social learning for collaborative natural resource management. *Society and Natural Resources*, 15, 309–326.

Siwela, A.H., Nyathi, C.B., & Naik, Y.S. (2010). A comparison of metal levels and antioxidant enzymes in freshwater snails, *Lymnaea natalensis*, exposed to sediment and water collected from Wright Dam and Lower Mguza Dam. *Ecotoxicology and Environmental Safety*, 73, 1728-1732.

Smolders, A.J.P; Lock, R.A.C, Velde, G., Hoyos, R.I.M., & Roelofs, J.G.M. (2003). Effects of mining activities on heavy metal concentrations in water, sediment, and macroinvertebrates in different reaches of the Pilcomayo River, South America. *Archives of Environmental Contamination and Toxicology*, 44, 314-323.

Sousa, R., Antunes, C., & Guilhermino, L. (2008a). Ecology of the invasive Asian clam *Corbicula fluminea* (Muller, 1774) in aquatic ecosystems: an overview. *Annales de Limnologie-International Journal of Limnology*, 44, 85-94.

Sousa, R., Dias, S.C., Guilhermino, L., & Antunes, C. (2008b). Minho River tidal freshwater wetlands: threats to faunal biodiversity. *Aquatic Biology*, 3, 237-250.

Sousa, R., Gutiérrez, J.L., & Aldridge, D.C. (2009) Non-indigenous invasive bivalves as ecosystem engineers. *Biological Invasions*, 11, 2367–2385.

Spray, S.L., & Mcglothlin, K.L. (2003). *Loss of Biodiversity. Exploring Environmental Challenges – a Multidiscilinary Approach*. Maryland: Rowman & Littlefield Publishers.

Stagg, B.C., Donkin, M.E., & Smith, A.M. (2014). Bryophytes for Beginners: The usability of a printed dichotomous key versus a multi-access computer-based key for bryophyte identification. *Journal of Biological Education*, DOI: 10.1080/00219266.2014.934900.

Strayer, D.L. (1999). Effects of alien species on freshwater mollusks in North America. *Journal of the North American Benthological Society*, 18, 74-98.

Strayer, D.L, & Malcom, H.M. (2007). Shell decay rates of native and alien freshwater bivalves and implications for habitat engineering. *Freshwater Biology*, 52, 1611-1617.

Strayer, D.L. (2010). Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology*, 55, 152-174.

Strayer, D.L. (2012). Eight questions about invasions and ecosystem functioning. *Ecology Letters*, 15, 1199-1210.

Svitok, M., Novikmec, M., Bitusik, P., Masa, B., Obona, J., Ocadlik, M., & Michalková, E. (2014). Benthic Communities of Low-Order Streams Affected by Acid Mine Drainages: A Case Study from Central Europe. *Water*, 6, 1312-1338.

Ter Braak, C.J.F. (1986). Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology*, 67, 1167-1179.

Ter Braak, C.J.F. (1995). Ordination. In R.H.G. Jongman, C.J.F. Ter Braak, & O.F.R. Tongeren (Eds.) *Data Analysis in Community and Landscape Ecology*. Cambridge: Cambridge University Press.

Vaikasas, S., Palaima, K, & Pliuraite, V. (2013). Influence of hydropower dams on the state of macroinvertebrates assemblages in the Virvyte river, Lithuania. Journal of Environmental Engineering and Landscape Management, 21, 305-315.

Van Damme, P., Hamel, C., Ayala, A. & Bervoits, L. (2008). Macroinvertebrate community response to acid mine drainage in rivers of the High Andes (Bolivia). *Environmental Pollution*, 156, 1061–1068.

Varol, M., Gökot, B., Bekleyen, A., & Sen, B. (2012). Spatial and temporal variations in surface water quality of the dam reservoirs in the Tigris River basin, Turkey. *Catena*, 92, 11-21.

Vaughn, C.C., & Hakenkamp, C.C. (2001). The functional role of burrowing bivalves in freshwater ecosystems. *Freshwater Biology*, 46, 1431-1446.

Verdonschot, P.F.M., & Nijboer, R.C. (2004). Testing the European stream typology of the Water Framework Directive for macroinvertebrates. *Hydrobiologia*, 516, 35-54.

Vidal, T., Santos, J.I., Marques, C.R., Pereira, J.L., Claro, M.T, Pereira, R., … Gonçalves, F. (2014). Resilience of the macroinvertebrate community os a small mountain river (Mau River, Portugal) subject to multiple stresses. *Marine and Freshwater Research*, 65, 1-12.

Voelz, N.J., McArthur, J.V., & Rader, R.B. (1998). Upstream mobility of the Asiatic clam *Corbicula fluminea*: Identifying potential dispersal agents. *Journal of Freshwater Ecology*, 13, 39–45.

Von der Ohe, P.C., & Liess, M. (2004). Relative sensitivity distribution of aquatic invertebrates to organic and metal compounds. *Environmemntal Toxicology and Chemistry*, 23, 150–156.

Walsh, C. (1997). A multivariate method for determining optimal subsample size in the analysis of macroinvertebrate samples. *Marine and Freshwater Research*, 48, 241-248.

WCD (2000). *Dams and Development: a New Framework for Decision-Making*. London: Earthscan Publication.

Werner, S. & Rothhaupt, K.O. (2007). Effects of the invasive bivalve *Corbicula fluminea* on settling juveniles and other benthic taxa. *Journal of the North American Benthological Society*, 26, 673-680.

Wernersson, A., Carere, M., Maggi, C., Tusil, P., Soldan, P., James, A., … Kase, R. (2015). The European technical report on aquatic effect-based monitoring tools under the water framework directive. *Environmental Sciences Europe*, 27, 1-11.

Wharfe, J.R., Taylor, K.S., & Montgomery, H.A.C. (1984). The growth of *Cladophora glomerata* in a river receiving sewage effluent. *Water Research*, 18, 971-979.

Whelch, E.B., Jacoby, J.M., Horner, R.R., & Seeley, M.R. (1988). Nuisance biomass levels of periphton algae in streams. *Hydrobiologia*, 157, 161-168.

Williams, E.H., Bunkley-Williams, L., Lilyestrom, C.G., & Ortiz-Corps, E.A.R. (2001). A review of recent introductions of aquatic invertebrates in Puerto Rico and implications for the management of nonindigenous species. *Caribbean Journal of Science,* 37, 246-251.

Wolkersdorfer, C., & Bowell, R. (2005). Contemporary Reviews of Mine Water Studies in Europe, Part 3. *Mine Water and the Environment*, 24, 58-76.

Xiaoyang, L., Shikiu, D., Qinghe, Z., & Shiliang, L. (2010). Impacts of Manwan Dam construction on aquatic habitat and community in Middle Reach of Lancang River. *Procedia Environmental Sciences*, 2, 706-712.

Zganec, K, Duric, P., Hudina, S., & Gottstein, S. (2013). Population and distribution changes of two coexisting river amphipods after the closure of a new large dam. *Limnologica*, 43, 460-468.

Ziv, G., Baran, E., Nam, S., Rodríguez-Iturbe, I., & Levin, S.A. (2012). Trading-off fish biodiversity, food security, and hydropower in the Mekong River Basin. *Proceedings of the National Academy of Sciences*, 109, 5609–5614.

CAPÍTULO II

Water quality and benthic macroinvertebrate community of river Sabor and its tributaries: regional and local perspectives

Abstract

Assessing the ecological status of waterbodies is necessary within the scope of the European Water Framework Directive (WFD), whose main goal is to attain or preserve good ecological quality in all waterbodies. As part of the ecological assessment, WFD recommends biomonitoring plans, which rely on bioindicators and biotic indexes. The analysis of the community structure of benthic macroinvertebrates represents an added value to detect these subtle modifications. The present study focused on the macroinvertebrate community of one of the few undisturbed rivers in northern Portugal (river Sabor). The objective was to comprehend the diversity and current ecological status of the river and some of its tributaries; also, we intended to assess potential impacts of the ongoing construction of a hydroelectric power station (with two dams). A sampling period from 2009 to 2013 was considered, each one represented by an annual sampling campaign during spring. In general, the ecological status in the study area was good or above, especially in the river's tributaries. Indeed, the small streams that flow into the main course are globally more diversified and rich. At some locations, the main river was poorly diversified, except when riffle habitats are found; this is a type of habitat that will be lost when the dam is filled. During the construction of the dams, some modifications were found in the macroinvertebrate community but they were only local and related with changes in flow; this produced some alterations in the ecological status. Community structure analysis also revealed inter-annual differences.

Key Words: Dam; Water Framework Directive; water quality; macroinvertebrate community; river Sabor; multivariate analysis

Introduction

Over the past century, lotic systems have been being seriously affected by anthropogenic influences (urban, industrial and agricultural activities, increasing exploitation of water resources, damming) and natural processes (changes in precipitation, erosion), which often resulted in the deterioration of the water quality, as well as in the overall degradation of the ecosystem (Varol, Gokot, Bekleyen, & Sen, 2012). Within the spirit of preserving freshwater resources, namely riverine ecosystems, the European Union has put in action a significant legislative approach, the Water Framework Directive (WFD), with the aim of attaining “good ecological status” in all freshwater systems by the end of 2015. However, a significant part of Europe’s freshwaters are at risk of not achieving this goal (EEA, 2015). There are multiple sources of impacts affecting European freshwater bodies that can contribute to this discouraging perspective, including contamination by nutrients (eutrophication) and xenobiotics, acidification, hydrological fluctuations (particularly drought), and damming (Hering et al., 2006; Dolédec & Statzner, 2008; Heathwaite, 2010). In addition, climate change is foreseen to increase contamination and water demand, therefore raising concerns on water scarcity (Heathwaite, 2010; IPCC, 2014), especially in vulnerable areas such as the Mediterranean region (García-Ruiz, lópez-Moreno, Vicente-Serrano, Lasanha-Martínez, 2011; IPCC, 2014).

The WFD brought a new ecocentric perspective to environmental assessments (Hering et al., 2003; INAG, 2008a), as it focuses on the assessment of biotic communities, going beyond the classical physical and chemical assessments, and it integrates this ecological information in the determination of the overall status of the waterbody. In this context, macroinvertebrate communities are particularly suited for the job, not only for their logistical advantages (Hellawell, 1986; Metcalfe-Smith, 1994), but mostly because their assemblage composition and diversity reflects long term past conditions (Blijswijk, Coimbra, & Graça, 2004), given their low mobility and relatively long life cycles (1-2 years). However, as stressed by Vidal et al. (2014), the current state of available information regarding environmental tolerance for most macroinvertebrate *taxa* was mostly derived from autoecological data on dissolved oxygen and nutrient level preferences (hence focused on organic pollution sensitivity), which brings some uncertainty to the indicator role of existing standard metrics when other types of stressors are involved, such as multiple-source pollution or physical changes to the habitat (Lorenz, Hering, Field, & Rolauffs, 2004; Davy-Bowker & Furse, 2006; Dunbar et al., 2010a, 2010b; Extence et al., 2013). Also, because rivers are spatially-structured dynamic systems, local *versus* regional influences and inter-annual differences add uncertainty to environmental assessments based on biotic communities. Thus, fine resolution analysis of riverine community structure and diversity may provide additional insight to the WFD approach (Vidal et al., 2014). Furthermore, the WFD classification system is somewhat reductionist, as it is based on the integration of simple univariate community metrics and biotic indices. Additional research on the sensitivity of fine community structure analysis tools and the WFD approach is still needed, particularly in real, multiple stressor scenarios.

Among the anthropogenic sources of environmental change, artificial modifications of river basins for irrigation or hydroelectric purposes (damming, deviation and canalization) are among the most extreme, and include heavy changes in topography, flow and discharge, and water residence time. Hydroelectric dam construction, for example, is still considered necessary to reduce external energy dependency or dependency on coal and gas combustion, to provide water for irrigation, to allow flood control, or for all of these purposes (Xiaoyan , Shikui, Qinghe, Shiliang, 2010). In Portugal, a governmental strategy has led to a new surge for the exploitation of rivers for hydroelectric production in this last decade, as a part of a national enterprise towards the development of the renewable energy market. This venture is in clear counter-current to the WFD objective of “good ecological status” for all freshwater masses, taking into consideration the complex changes and predicted impacts that are expected in riverine ecosystems throughout the sequential phases of river damming, including construction, filling and operation. Indeed, numerous studies have documented adverse, often unwanted, consequences on hydrological connectivity, flow regulation, sediment delivery, biodiversity (Grill, Dallaire, Chouinard, Sindorf, & Lehner, 2014) and habitat integrity (Zganec, Duric, Hudina, & Gottstein, 2013), both locally and regionally. More specifically, dams and the resulting reservoirs affect the ecological framework by challenging both the terrestrial habitat, including habitat area decrease and alteration of its spatial configuration, and the aquatic habitat, including multiple hydrological, physical (e.g. surface water temperature) and water quality changes (Xiaoyan et al., 2010). Moreover, dams across rivers create physical barriers of natural water flow, with the consequent degradation and fragmentation of populations (Looy, Tormos, & Souchon, 2014).

Sabor river, a tributary of one of the largest Iberian rivers (Douro), was targeted for hydroelectric exploitation, via a large-scale pumped-storage plant (with two dams) whose construction began in the summer of 2008. After some technical problems, the upstream and downstream reservoirs started filling in 2014. Given the proximity of the dams to the river's confluence with the Douro, most of the predicted impacts are located in the lower Sabor valley, a Natura 2000 site (special protection area PTZPE0037 and site of community importance PTCON0021) renowned for being one of the last "wild" regions in Portugal (Freitas & Horta, 2003; Paterson,Araújo, Berry, Piper, Rounsevell, 2008; Melo, 2009). This reputation stems from the local biodiversity and high levels of endemism, as well as its important biogeographic role (see references in Paterson et al., 2008), although most of these data are scattered throughout academic theses or technical reports (e.g. Hoelzer, 2003). Apart from the social and economic aspects of the project, and the ongoing debate about its (un)sustainability (Melo, 2009; Jackson, 2011), this man-made change to a putatively non-impacted aquatic system provided an opportunity to look at the problem from the water quality perspective.

Bearing this in mind, this study intended to evaluate the diversity and ecological status of the lower Sabor river and some of its tributaries prior to the filling of the reservoir, by using the WFD approach based on macroinvertebrate community. Our objective was four-fold: a) to evaluate the putative non-impacted reputation of the lower Sabor, in terms of its riverine assemblages and water quality; b) to assess the spatial (local and regional) patterns and temporal heterogeneity along the hydrographic basin; c) to establish a baseline reference condition prior to the upcoming changes in hydrology, hydromorphology and connectivity due to reservoir filling; d) to assess the impacts of the construction of the dams on the macroinvertebrate community and ecological status. To accomplish this, a sampling campaign was carried out from 2009 to 2013, merging three approaches: (i) physical, chemical and hydromorphological measurements, as well as other supporting environmental variables, (ii) assessment of the structural changes in the macroinvertebrate community through time and space (iii) evaluation of the sensitivity of the WFD approach to detect ecologically important community changes.

Material and Methods

Study area and sampling strategy

Sabor river belongs to Douro basin, in northeastern Portugal. Its headwater is located in Serra de Parada, Spain, and it flows with NE-SW orientation for an extension of approximately 131 km until its mouth in Douro river. It has an area of approximately 3981 km^2 in Portuguese territory. The population density and industrial activity are not very high, resulting in an overall low human influence on the aquatic ecosystems in the region (INE, 2012). Sabor river is classified by INAG (2008b) as a Type N2 river (medium-large rivers of Alto Douro) and its hydrographic basin is composed by several small tributaries (Type N3 – small dimension rivers of Alto Douro), embedded in a heterogeneous landscape. Regionally, Mediterranean climate typically produces dry summers (and often water scarcity) and rainy winters.

Sampling campaigns were carried out annually during a five-year period (2009-2013) in the spring (from April to June). Fifteen sampling sites in the main river (Sab1-Sab15) were defined in order to cover the river segment that will be potentially affected by dam construction and flooding (Sab6-Sab15), as well as a “control” upstream segment with five sampling sites (Sab1-Sab5). Additionally, twenty-two sites along the river’s tributaries were also sampled (Figure 1; Table 1). These watercourses are mainly low order streams, presenting narrow water channels and riparian vegetation that promotes different degrees of shading. Unlike the main river section, which is fairly homogeneous throughout its course, the tributaries are hydromorphologically diverse.

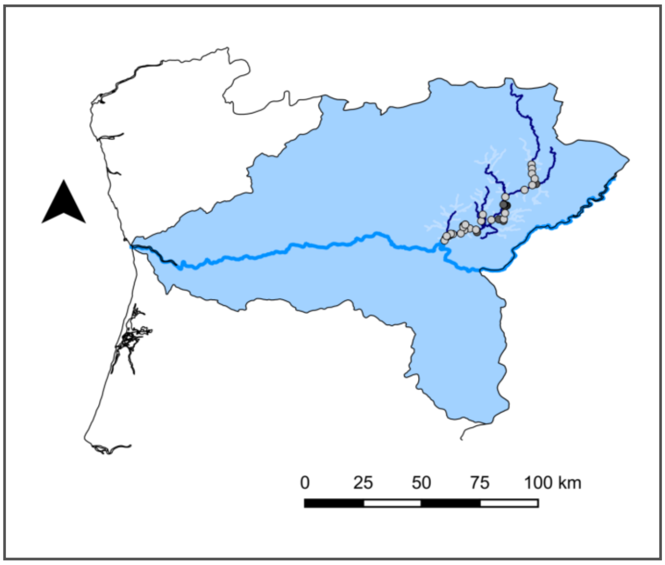


Fig. 1. Location of Sabor river and its tributaries in the Douro river basin, showing sampling sites.

Table 1. Information about sampling sites (along upstream-downstream gradient) and sampling year (Main course – MC; Tributary - T).

Site	River	GPS coord.	Location	2009	2010	2011	2012	2013
Sab1	Sabor river	41°33'32.2"N 6°40'30.3"W	MC		x	x	x	x
Sab2	Sabor river	41°32'15.7"N 6°40'22.7"W	MC		x	x	x	x
Sab3	Sabor river	41°30'46.2"N 6°40'23.5"W	MC		x	x	x	x
Sab4	Sabor river	41°29'16.1"N 6°39'34.0"W	MC		x	x	x	x
Mac1	Maçãs river	41º27.692'N 6º39.119'W	MC	x	x			
Mac2	Maçãs river	41º27.568'N 6º39.914'W	MC	x	x			
Sab5	Sabor river	41°27'20.9"N 6°40'17.6"W	MC		x	x	x	x
Sab6	Sabor river	41°25'55.3"N 6°42'36.9"W	MC		x	x	x	x
Sab7	Sabor river	41°23'49.2"N	MC	x	x	x	x	x

		6°48'21.6"W						
Poi1	Poio stream	41º21.516'N 6º48.699'W	T	x	x	x		x
Poi2	Poio stream	41º21.490'N 6º48.911W	T	x	x			
Jun1	Juncainhos stream	41º21.209'N 6º47.909'W	T	x	x			
Jun2	Juncainhos stream	41º21.112'N 6º48.145'W	T	x	x			
Jun3	Juncainhos stream	41º21.112'N 6º48.548'W	T	x	x			
Sou1	Souto stream	41º18.912'N 6º47.346'W	T	x	x	x		x
Sou2	Souto stream	41°18'57.0"N 6°48'26.5"W	T	x	x	x	x	x
Spe1	S. Pedro stream	41°17'09.4"N 6°48'49.4"W	T	x	x	x	x	x
Spe2	S. Pedro stream	41º16.592'N 6º48.931'W	T	x	x			
Spe3	S. Pedro stream	41º17.096'N 6º50.312'W	T	x	x			
Sab8	Sabor river	41°16'55.1"N 6°52'31.9"W	MC	x	x	x	x	x
Zac	Zacarias stream	41°18'31.6"N 6°55'06.5"W	T		x		x	x
Cal	Calvário stream	41°16'33.9"N 6°55'34.2"W	T				x	x
Moi1	Moinhos stream	41º12.991'N 6º56.492'W	T	x	x			
Moi2	Moinhos stream	41°13'57.5"N 6°57'08.2"W	T	x	x	x	x	x
Sab9	Sabor river	41°14'19.9"N 6°59'00.1"W	MC	x	x	x	x	x
Rel1	Relvas stream	41º16.347'N 6º59.037'W	T	x	x			
Rel2	Relvas stream	41°15'28.7"N 7°00'20.8"W	T	x	x	x	x	x
Xed1	Xedal stream	41°15'06.2"N	T	x	x	x		x

		7°00'55.9"W						
Xed2	Xedal stream	41°14'52.2"N 7°00'53.0"W	T	x	x	x		
Xed3	Xedal stream	41°14'45.2"N 7°00'56.2"W	T	x	x	x	x	x
Sab10A	Sabor river	41°14'18.1"N 7°00'19.9"W	MC	x	x	x		
Sab10B	Sabor river	41°13'49.9"N 7°00'35.4"W	MC				x	x
Sab11	Sabor river	41°12'51.2"N 7°01'40.5"W	MC		x	x	x	x
Sab12	Sabor river	41°12'38.8"N 7°04'05.3"W	MC	x	x	x	x	x
Sab13A	Sabor river	41°12'14.0"N 7°05'07.2"W	MC	x	x	x		
Sab13B	Sabor river	41°12'45.0"N 7°04'40.0"W	MC				x	x
Vil	Vilariça stream	41°12'03.0"N 7°05'54.8"W	T			x	x	x
Sab14A	Sabor river	41°11'22.2"N 7°06'14.4"W	MC	x	x	x		
Sab14B	Sabor river	41°11'55.3"N 7°05'54.6"W	MC				x	x
Sab15	Sabor river	41°10'42.8"N 7°06'32.6"W	MC		x	x	x	x

Physicochemical analysis

Dissolved oxygen (‘oxi’), pH and conductivity (‘cond’) were measured locally at each sampling site with a portable multiparameter sensor (WTW Multi 3430 SET F). These variables were complemented with available data on temperature and precipitation: average values in the month of sampling, previous month and previous 6 months (‘avg temp’, ‘avg temp 1 month’, ‘avg temp 6 months’; and ‘avg precip, ‘avg precip 1 month’, ‘avg precip 6 months’).

A snapshot characterisation of the general hydromorphological features of each site was carried out *in situ,* by recording flow (on an ordinal scale from 0-4), shade (on an ordinal scale from 0-3), width (on an ordinal scale from 1-5) and depth (on an ordinal scale from 1-3) of the channel, transparency (‘transp’, on an ordinal scale from 1-3), characterization of substrate (‘fines’, ‘sand’, ‘gravel’, ‘stones’, ‘rocks’), continuity of riparian gallery (‘rip gall’, on an ordinal scale from 0-4) and its constitution (macrophytes – ‘macroph’, briophytes – ‘brioph’, herbaceous – ‘herb’, ‘short bush’, tall bush’, ‘short tree’, ‘tall tree’), debris present in the water and obstacles to flow (‘fallen trees’, ‘logs and leaves’, ‘dam’, ‘construction’, water withdrawal – ‘water wd’).

Latitude and longitude of each site was recorded *in situ* and a map was built with QuantumGis software (QGIS Development Team, 2015). Distance of each sampling site to the main river course and to its mouth was measured in the study area map using ImageJ 1.48v (Abràmoff et al., 2004).

Physical, chemical and supporting environmental variables data (see above) were compiled into three multivariate matrices: (i) environmental matrix, including records of temperature, precipitation and water physico-chemical parameters; (ii) hydromorphological matrix, comprising data on the morphology, vegetation and disturbance of the channel and banks; (iii) spatial matrix, including geographical and distance data of sampling sites.

Macroinvertebrate sampling and identification

Macroinvertebrates were collected at each sampling site following a standard methodology (INAG, 2008a), using a kick sampling technique. At each site, a hand net (500 µm pore size, square frame 0.30 x 0.30 m) was used to sample the most representative microhabitats, by applying a similar sampling effort per site (six 1-m transects, pooled into one composite sample, and distributed proportionally across the existing microhabitats) (Hering et al., 2003; INAG, 2008a). The collected benthic invertebrate samples were preserved with ethanol (70-80%) in the field. At the laboratory, preserved samples were transferred into a sieve (500 µm pore size) and gently washed with tapwater. They were then sorted out and organisms were stored in 70% ethanol. All collected organisms were identified with a stereomicroscope to the lowest practical taxonomical level, generally the family (or genus, when possible) using adequate dichotomous identification keys (Hynes, 1993; Tachet, 2000; Wallace, Wallace, Philipson, 2003; Edington & Hildrew, 2005;

Sundermann, Lohse, Beck, Haase, 2007; Serra et al., 2009; Elliott & Humpesch, 2010; Pawley, Dobson, Fletcher, 2011).

Data analysis: WFD approach

Macroinvertebrate data (using family as the lowest taxonomical level) was analysed using the following community metrics: richness (total number of families, S), Shannon's diversity index (H') and Pielou's equitability index (J'). Four biotic metrics were also calculated: EPT *taxa* (number of *taxa* belonging to orders Ephemeroptera, Plecoptera and Trichoptera), ETD (sum of abundances of organisms belonging to families Heptageniidae, Ephemeridae, Brachycentridae, Goeridae, Odontoceridae, Limnephilidae, Polycentropodidae, Athericidae, Dixidae, Dolichopodidae, Empididae, Stratiomyidae), as well as the Iberian BMWP (Alba-Tercedor & Sánchez-Ortega, 1988; Chapman & Jackson, 1996) and the resulting normalised average score per taxon (IASPT). These metrics were integrated in the calculation of the North Invertebrate Portuguese Index ($IPtI_N$) (INAG, 2009), a multimetric index equivalent to ICM 7/STAR (Munné & Prat, 2009):

$IPtI_N = (0.25 \times S) + (0.15 \times EPT) + (0.1 \times J') + (0.3 \times (IASPT - 2)) + (0.2 \times \log(Sel.\ ETD + 1))$.

Prior to computing the index, the community and biotic metrics were normalised by dividing each of them by their corresponding reference value; the resulting $IPtI_N$ values were also divided by their respective reference values. This two-step normalisation procedure (INAG, 2009) resulted in the derivation of Ecological Quality Ratios (EQRs) for each sampled site (INAG, 2009). Reference values for the community metrics, biotic indices, and $IPtI_N$ values were set after a pan-European intercalibration exercise (JRC, 2009), and are available in official guidance documents (INAG, 2009) for each river type (for the purpose of this study, reference values for Types N2 or N3 rivers were used – see *Study area and sampling strategy*).

To aid in interpretation, ecological indices (richness, diversity, EPT, IASPT, $IPtI_N$, and EQR) were analysed with non-parametric statistical methods using R software (R Core Development Team, 2015), separately for each year. First, Kendall's tau (τ) was calculated (using the *corr.test()* function of 'psych' package;

Revelle, 2016) as a measure of association of each index with distance to mouth (from the spatial data matrix) to assess whether these metrics were correlated to the upstream-downstream gradient. Because these represented multiple related hypotheses, *p*-values were corrected by controlling false discovery rate (Benjamini & Hochberg, 1995), a feature that is optional with *corr.test()* function. Second, we tested for differences between the metrics in main course *versus* tributaries with Kruskal-Wallis tests (corrected for ties), using the *kruskal.test ()* function of "stats" package (R Core Development Team, 2015). A significance level of 0.05 was used in all analyses.

Data analysis: multivariate approach

Benthic invertebrate abundance data (using the lowest possible taxonomical resolution) was compiled as a multivariate matrix, which was normalized across years. In doubt or when taxonomical resolution varied (due to logistical or human constraints), similar taxa were pooled into a larger taxonomical category (usually family, with the exception of Oligochaeta - class). Taxa occurring in less than 5% of the samples were removed, independently of their abundance, as a precautionary measure to minimize their potentially inflated influence in ordination. Additionally, abundance data were log-transformed, to prevent excess weight of highly abundant species.

Each environmental subset (see *Physical, chemical and supporting environmental variables*) was used as a potential explanatory data matrix, after removing non-significant variables with a manual forward selection procedure (Monte-Carlo permutation test, $P \leq 0.05$). Canonical Correspondence Analysis (CCA) was then run (ter Braak, 1995) to perceive the amount of macroinvertebrate variation caused by the global explanatory dataset and each individual subset (environmental, hydromorphological and spatial matrices) (see other examples in Castro, Antunes, Pereira, Soares, & Gonçalves, 2005). To explore inter-annual differences, this analysis was also performed for each year separately. Data obtained from these analyses were plotted in a bidimensional space in species *versus* environmental variables and samples *versus* environmental variables plots.

All multivariate analyses were run in CANOCO v.4.5 (Braak & Smilauer, 1998) and all models were tested for significance ($P \leq 0.05$) using a Monte-Carlo unrestricted permutation test.

Results and Discussion

Sabor river was expected to suffer the effects of damming, either at specific locations or along its extension. However, the ecological status in the study area, in general, revealed that good water quality was found, especially in the river's tributaries. This was verified both with the biotic indices and community structure approaches, which allowed to identify localized (in space and in time) impacts on the macroinvertebrate assemblage. Although useful and recommended within the WFD scope, the use of biotic indices was not as discriminating as the community structure analysis. The latter approach explored spatial and temporal trends with finer resolution, allowing a more detailed analysis of the species succession. Moreover, this may aid in identifying the causal nature of the observed impacts (Varol et al., 2012).

Macroinvertebrate communities are affected by many direct and indirect factors (Poff et al., 2010). For example, climate directly influences vegetation growth, which conditions organic matter availability and decomposition rate, exerting influence on feeding traits of invertebrates and, consequently, community structure (as described by Aschonitis et al., 2016). There are a number of macroinvertebrates species that can be found in a river zone indicating the condition of the water body. Many of the recent studies have looked into three orders of insects that are distinctly sensitive to habitat disturbance: ephemeroptera (mayflies), plecoptera (stoneflies) and tricoptera (caddisflies) (e.g., Ancog, Andrade, Miasco, Ortiz, 2010).

A total of 88 macroinvertebrate *taxa* were identified throughout the five years of sampling, belonging to a total of 65 families. The full list of taxa can be found in Supplementary material (Tables S1-S5).

Community metrics and ecological status

Ecological indices indicated that interannual differences were present but there was also large spatial heterogeneity. No significant correlations were found with distance to mouth for any variable (Table 2), with no obvious upstream-downstream gradient shown in terms of richness, diversity, bioindicator value of the invertebrate community or ecological status. However, some differences between main course *versus* tributary sampling sites were observed in calculated metrics (Figure 2).

Table 2. Correlation between ecological indices and upstream-downstream gradient (distance to mouth).

Correlation	Richness	Diversity	EPT	IASPT	$IPtI_N$	EQR
Year 2009						
Kendall's τ	0.06	0.01	0.08	0.10	0.05	0.05
***p* value**	0.84	0.96	0.84	0.83	0.84	0.84
Year 2010						
Kendall's τ	0.11	0.07	0.11	-0.01	0.08	0.08
***p* value**	0.66	0.73	0.66	0.97	0.70	0.70
Year 2011						
Kendall's τ	0.17	0.36	0.37	0.37	0.41	0.41
***p* value**	0.44	0.10	0.10	0.09	0.06	0.06
Year 2012						
Kendall's τ	0.02	0.02	0.06	0.06	0.03	0.03
***p* value**	0.94	0.94	0.94	0.94	0.94	0.94
Year 2013						
Kendall's τ	0.09	-0.11	0.27	0.36	0.09	0.09
***p* value**	0.66	0.66	0.28	0.15	0.66	0.66

Fig. 2. Ecological indices across sampling sites for years 2009-2013.

In general, macroinvertebrate communities from the tributaries were richer (had more taxa), more diverse and had a higher EPT score (Table 3; Figure 2); this was mostly observed in 2009-2010. Water quality indices (IASPt and $IPtI_N$) did not vary between main course and tributaries, except in 2010 (with a slightly higher score in tributary sampling sites). Ecological quality ratios (EQR) were variable spatially and interannually, although without an obvious upstream-downstream gradient (as referred above) or pronounced differences between main course and tributaries (except in 2010).

Table 3. Summary table for the test of differences between main course and tributaries. Significant values are highlighted in bold.

Correlation	Richness	Diversity	EPT	IASPT	$IPtI_N$	EQR
Year 2009						
Kruskal-Wallis *H*	7.89	6.01	5.67	3.56	3.46	3.46
d.f.	1	1	1	1	1	1
***p* value**	**0.005**	**0.014**	**0.017**	0.059	0.063	0.063
Year 2010						
Kruskal-Wallis *H*	14.2	15.0	10.2	11.1	11.3	11.3
d.f.	1	1	1	1	1	1
***p* value**	**< 0.001**	**< 0.001**	**0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Year 2011						
Kruskal-Wallis *H*	10.1	0.257	0.130	0.108	0.0009	0.0009
d.f.	1	1	1	1	1	1
***p* value**	**0.002**	0.612	0.719	0.743	0.976	0.976
Year 2012						
Kruskal-Wallis *H*	2.03	1.35	0.422	0.761	1.50	1.50
d.f.	1	1	1	1	1	1
***p* value**	0.154	0.245	0.516	0.383	0.220	0.220
Year 2013						
Kruskal-Wallis *H*	6.90	2.51	0.0336	0.491	0.114	0.114
d.f.	1	1	1	1	1	1
***p* value**	**0.009**	0.113	0.855	0.484	0.736	0.736

Overall, sampled sites were scored as having good or moderate ecological status, irrespective of their position in the river continuum or stream order (main course *versus* tributaries; Figures 3-7).

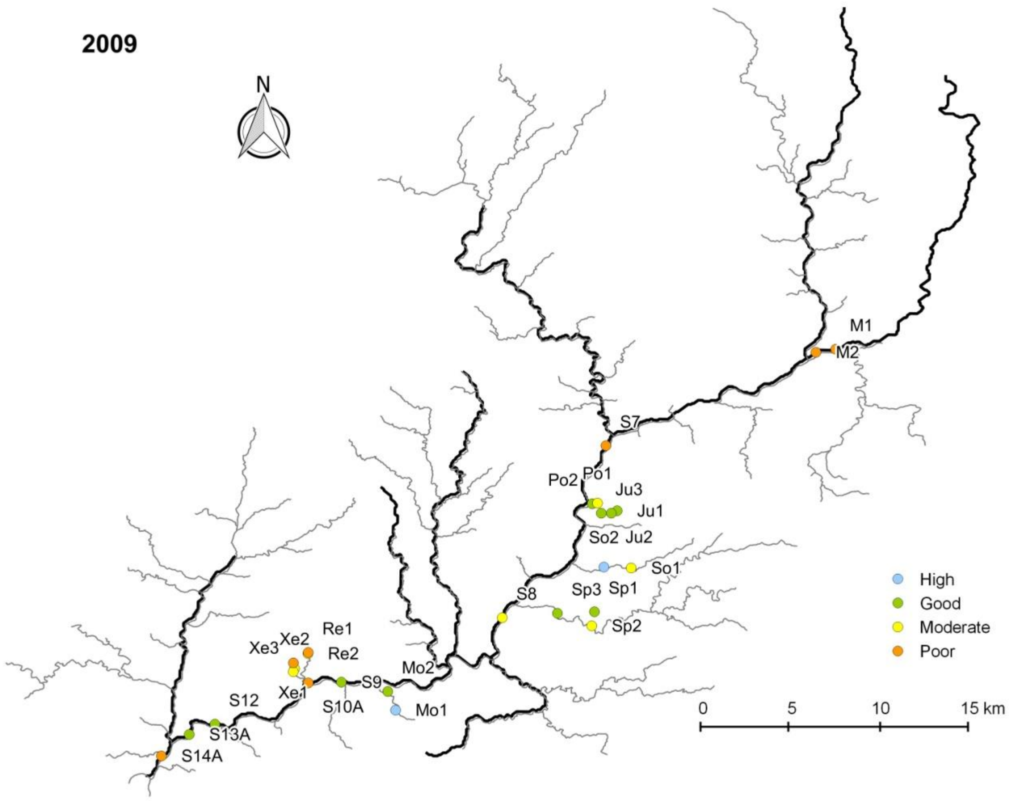


Fig. 3. Sampling sites in 2009 and associated ecological status (colour code).

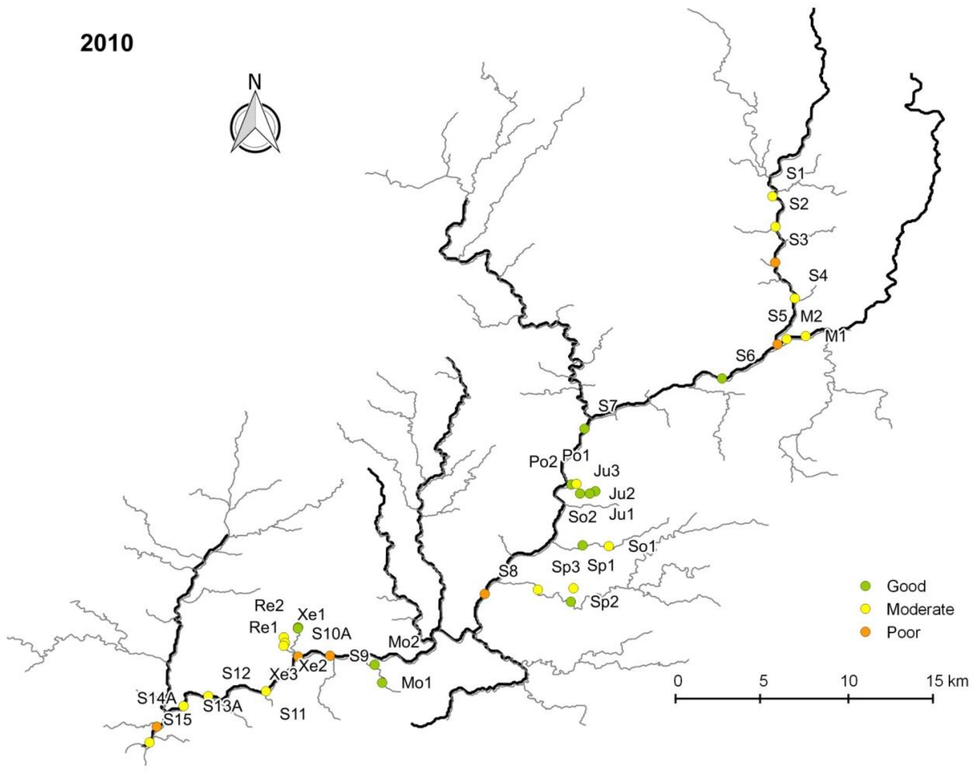


Fig.4. Sampling sites in 2010 and associated ecological status (colour code).

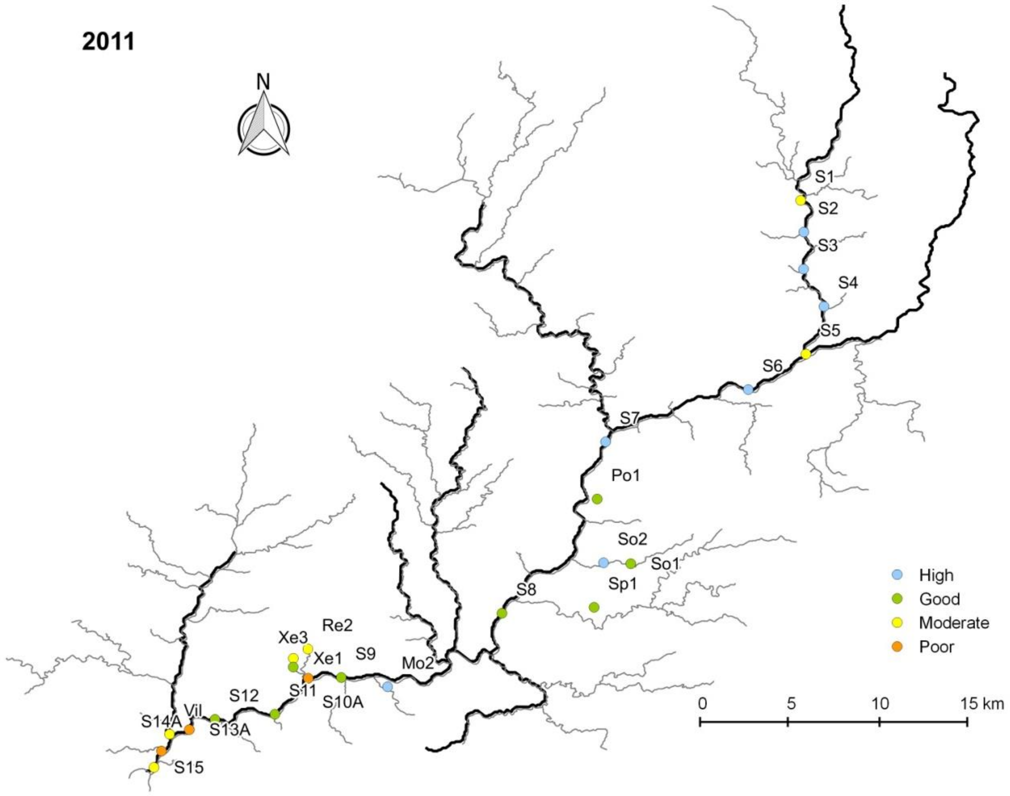


Fig. 5. Sampling sites in 2011 and associated ecological status (colour code).

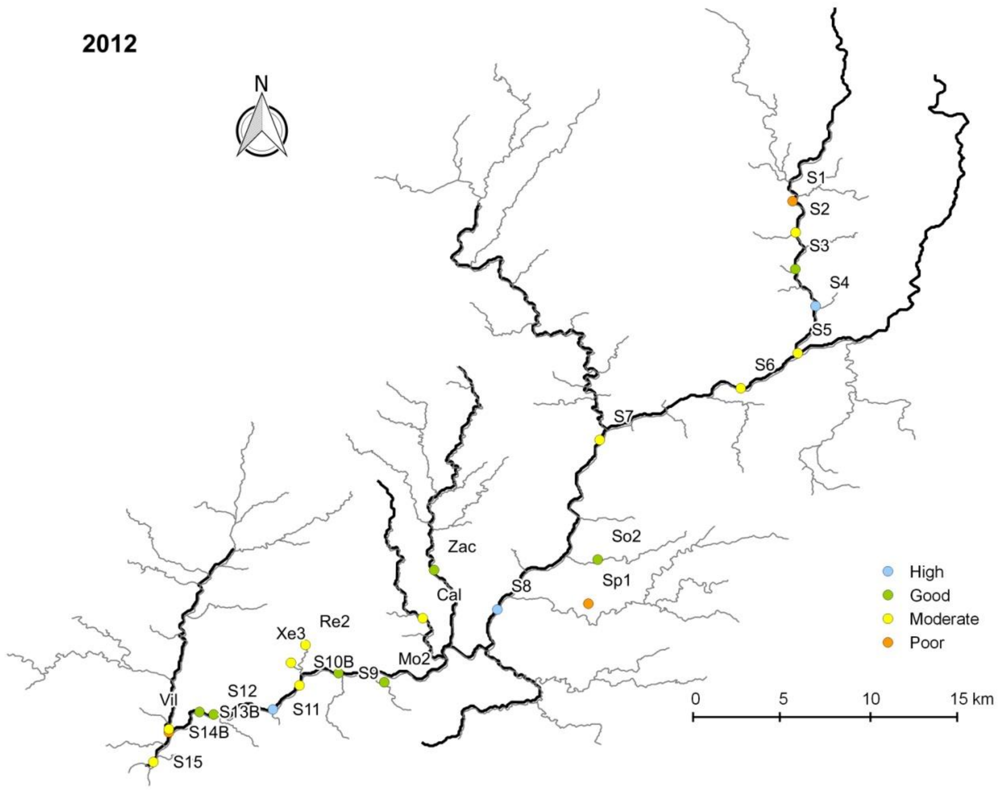


Fig. 6. Sampling sites in 2012 and associated ecological status (colour code).

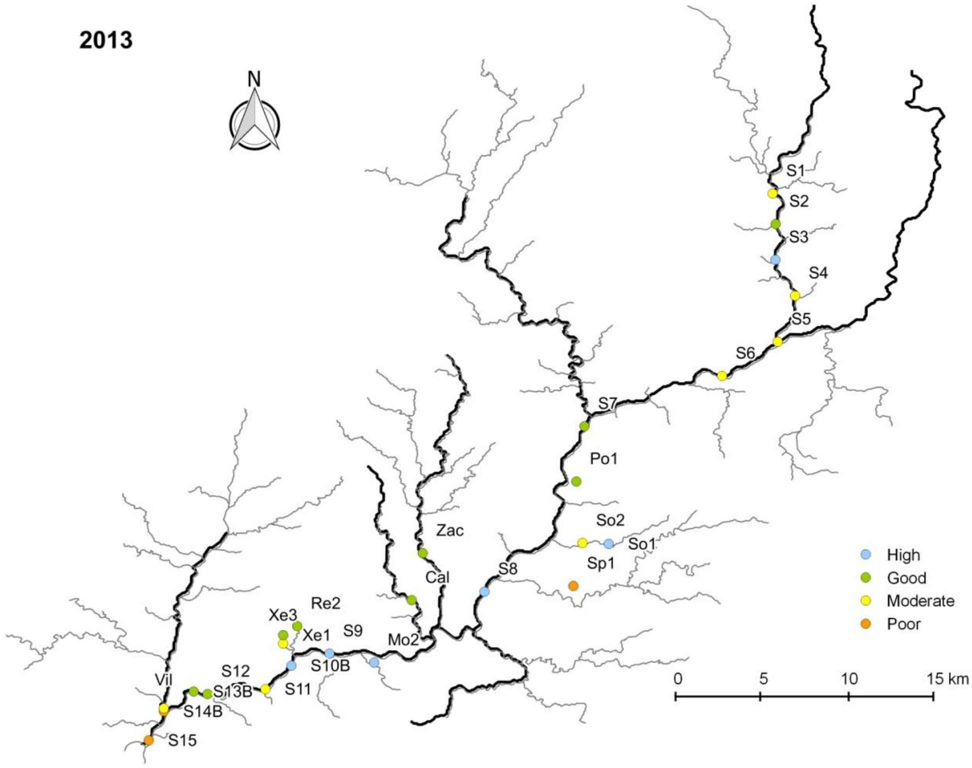


Fig. 7. Sampling sites in 2013 and associated ecological status (colour code).

A few sites attained a high ecological status whilst others were scored as poor, although this varied throughout the sampling period (Table 4). Sampling sites Sab10A, Sab14A and Sab14B consistently had poor ecological status; also, site Spe1 showed a pronounced decrease in ecological status through time scoring twice as 'Poor' in 2012-2013. Table 4 adds further detail to the data above, showing the ecological status of the sampling sites after integrating the records of the five years. As previously said, most sites had moderate to good ecological status. Sites with poor quality were located either in the lower (Sab10A, Sab14A, Sab14B and Sab15) or in the upper (Sab1, Sab5, Mac1 and Mac2) reaches of the main course. Of all sampling sites, Moi1 and Moi2 (Moinhos stream) had the best ecological quality, along with Sou2. Five other sites located in small tributaries had good ecological quality; thus, eight out of 20 sampling sites located in tributaries had an overall good ecological status. Only four of the 20 sampling sites located in the main course had good ecological quality (Sab3, Sab4, Sab10B and Sab13B).

Table 4. Ecological status of each sampling site in a temporal (five years) perspective: numbers on columns 2-6 represent the number of records for each site within each category (transformed into a 0-4 scale); 'Global' represents the average score (0-4) of the site pooling all years.

Sites	Bad (0)	Poor (1)	Moderate (2)	Good (3)	High (4)	Global	Boundaries
Sab10A	0	3	0	0	0	1	
Sab14A	0	3	0	0	0	1	
Sab14B	0	2	0	0	0	1	
Mac1	0	1	1	0	0	1.5	
Mac2	0	1	1	0	0	1.5	
Sab1	0	1	3	0	0	1.75	
Sab15	0	1	3	0	0	1.75	
Sab5	0	1	3	0	0	1.75	↑ less than moderate
Rel1	0	1	0	1	0	2	↓ moderate to good
Sab13A	0	1	1	1	0	2	
Spe1	0	2	1	2	0	2	
Vil	0	0	3	0	0	2	
Xed1	0	1	2	1	0	2	
Xed2	0	0	2	0	0	2	
Xed3	0	0	4	1	0	2.2	
Rel2	0	0	3	2	0	2.4	
Cal	0	0	1	1	0	2.5	
Poi1	0	0	2	2	0	2.5	
Spe2	0	0	1	1	0	2.5	
Spe3	0	0	1	1	0	2.5	
Sab7	0	1	1	2	1	2.6	
Sab11	0	0	2	1	1	2.75	
Sab2	0	0	2	1	1	2.75	
Sab6	0	0	2	1	1	2.75	
Sou1	0	0	2	1	1	2.75	
Sab12	0	0	1	4	0	2.8	
Sab8	0	1	1	1	2	2.8	
Sab9	0	1	0	3	1	2.8	↑ moderate to good
Jun1	0	0	0	2	0	3	↓ good or more
Jun2	0	0	0	2	0	3	
Jun3	0	0	0	2	0	3	
Poi2	0	0	0	2	0	3	

Sab10B	0	0	1	0	1	3
Sab13B	0	0	0	2	0	3
Sab3	0	1	0	1	2	3
Sab4	0	0	2	0	2	3
Zac	0	0	0	2	0	3
Sou2	0	0	1	2	2	3.2
Moi2	0	0	0	3	2	3.4
Moi1	0	0	0	1	1	3.5

Community structure

A global CCA on macroinvertebrate taxa, integrating all years, showed a clear association of sampling sites from each year, documenting a strong inter-annual variation in terms of species composition and abundance (Figure 8). Generally, sample scores from 2009 and 2010 appeared to be superimposed, indicating a high degree of similarity between the macroinvertebrate community in these two years. The same is true for 2012 and 2013, but this cluster is clearly separated from the previous. Sample scores from 2011 form a distinct cluster from the previous two. This justifies a detailed analysis of each year individually.

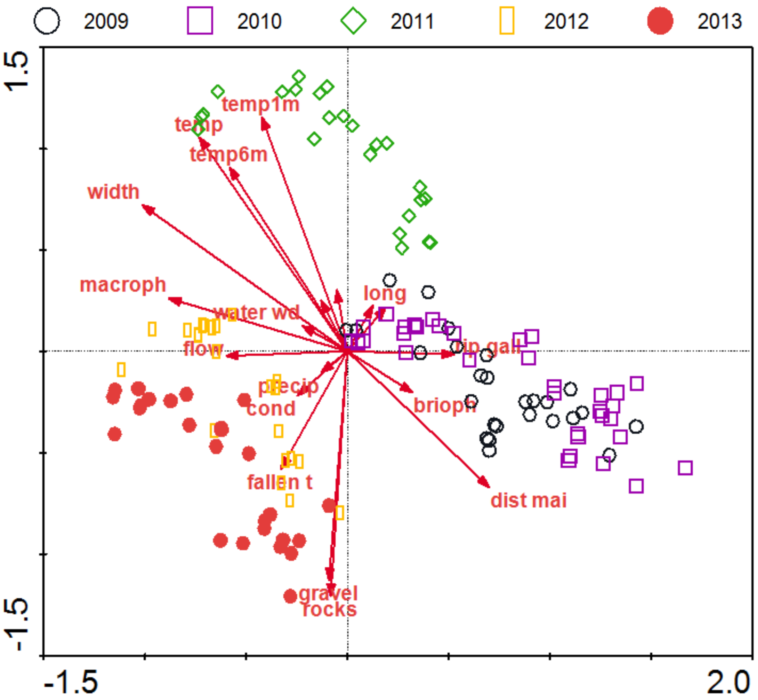


Fig. 8. Global CCA biplot integrating the sampling period 2009-2013. Symbols represent sampling sites and arrows represent explanatory variables.

Overall, CCAs conducted for each year (Figures 9-13) revealed a limited scatter of samples in ordination space demonstrating a fairly homogeneous macroinvertebrate community at the hydrographic basin level. However, this changed on a yearly basis, with a less pronounced homogeneity in 2009. Nevertheless, some degree of heterogeneity was observed, mainly attributable to channel width, followed in importance by distance to main course and conductivity. To a lesser extent, flow, riparian gallery and transparency were also important drivers of the macroinvertebrate community. Within the observed heterogeneity, a clear and consistent separation was observed between sites located in tributaries *versus* main course (Figures 9-13). This separation is associated with the observed gradient in terms of channel width (sites within tributaries are consistently narrower) and distance to main course. Although compositional differences between tributaries and main course varied across years, a detailed analysis of the dataset revealed some *taxa* that were consistently associated the main course, namely *Ephoron*, *Chironomini* and *Caenis*. All these taxa have low IBMWP scores because they are tolerant to pollution, being an importants organisms to explain the distribution of the sampling sites located in the main river in all years, with the exception for 2010. Main river were, also, mostly constituted by shredders (and predators) with low IBMWP score which indicates its bad condition of the main river. However, tributaries tended to present exclusive or almost exclusive taxa, which were not present in the main course; this can be seen by the distribution of species scores in the diagram, namely Chloroperlidae, Corixidae, Libellulidae, Polycentropodidae and Stratyomiidae. The presence of taxa exclusive to tributaries justifies the above observations of increased diversity and richness in these smaller, and more biodiverse systems.

Another visible pattern in the CCAs was the segregation of the most downstream sampling sites (Sab14 and Sab15) in all years except 2013. This is an indicator of the homogenization of the river, with the most recent year lacking differentiation of the most downstream portion, as opposed to what happened in the previous years. The macroinvertebrate community in these sites was dominated by *Micronecta* sp., *Atyaephyra*, and other taxa typical of low-flow waters (Gerridae, Naucoridae); this can be observed by the association of the respective species scores and Sab14 and Sab15 sample scores. *Atyaephyra* sp. Is also associated with the river's sampling sites distribution in general, which is very tolerant to variations of temperature and salinity and easily occupies niches left empty by more sensitive invertebrates. Not so clear but also worth the mentioning is the case of Vil (located in Vilariça stream), which, although located on a stream, was scattered in the graphs consistently close to main course sampling sites, illustrating it as a transition site, largely influenced by the main course natural characteristics.

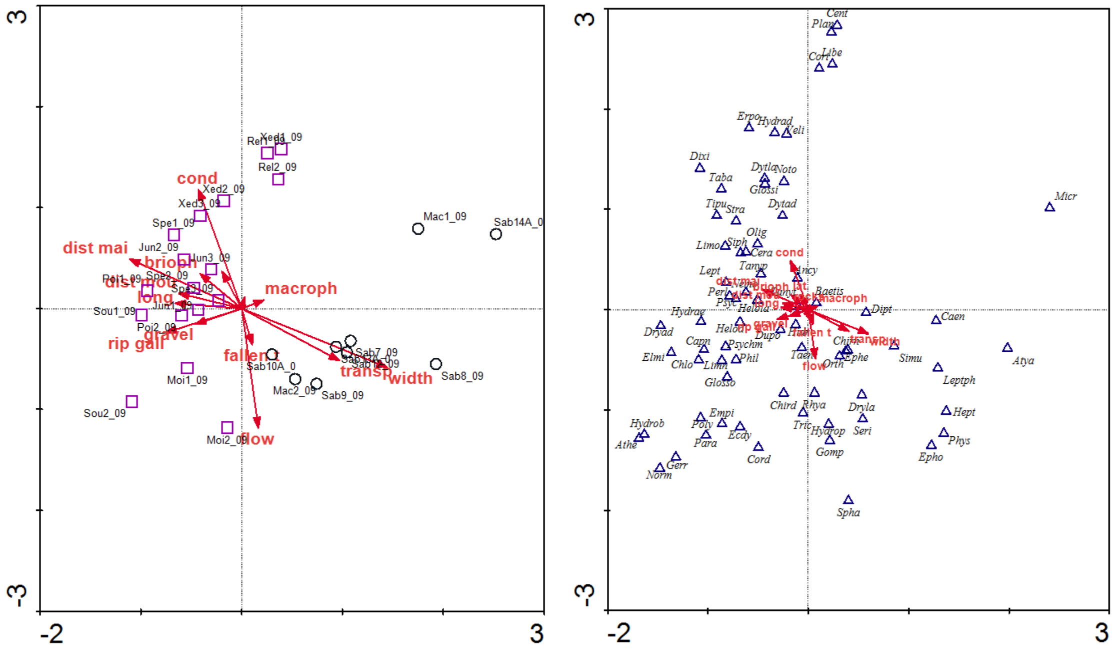


Fig. 9. CCA biplots for 2009 data. Circles (main course of the river) and squares (tributaries) stand for sampling sites, whilst triangles represent species (for taxa abbreviations, see Table S1). Arrows represent explanatory variables in both diagrams.

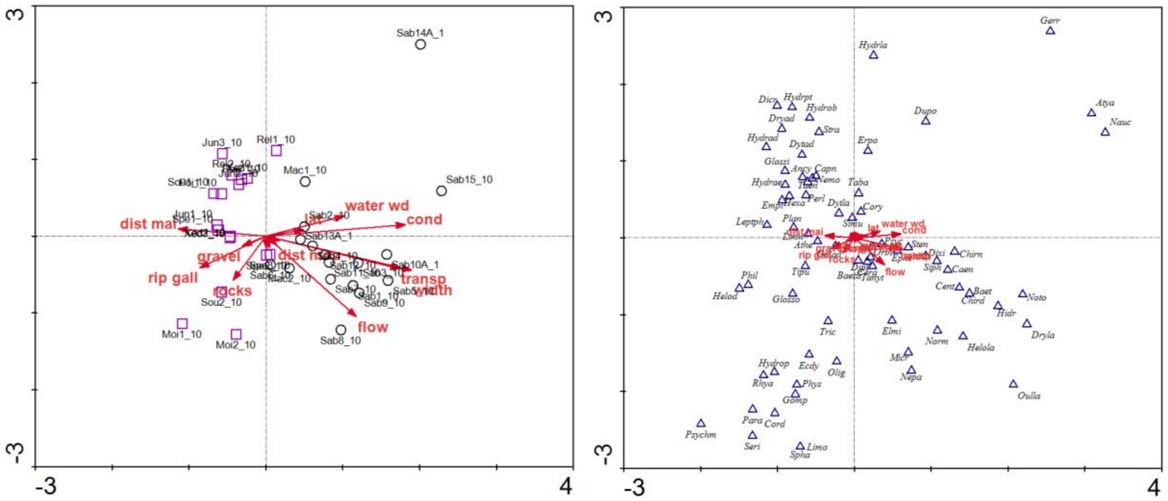


Fig. 10. CCA biplots for 2010 data. Circles (main course of the river) and squares (tributaries) stand for sampling sites, whilst triangles represent species (for taxa abbreviations, see Table S1). Arrows represent explanatory variables in both diagrams.

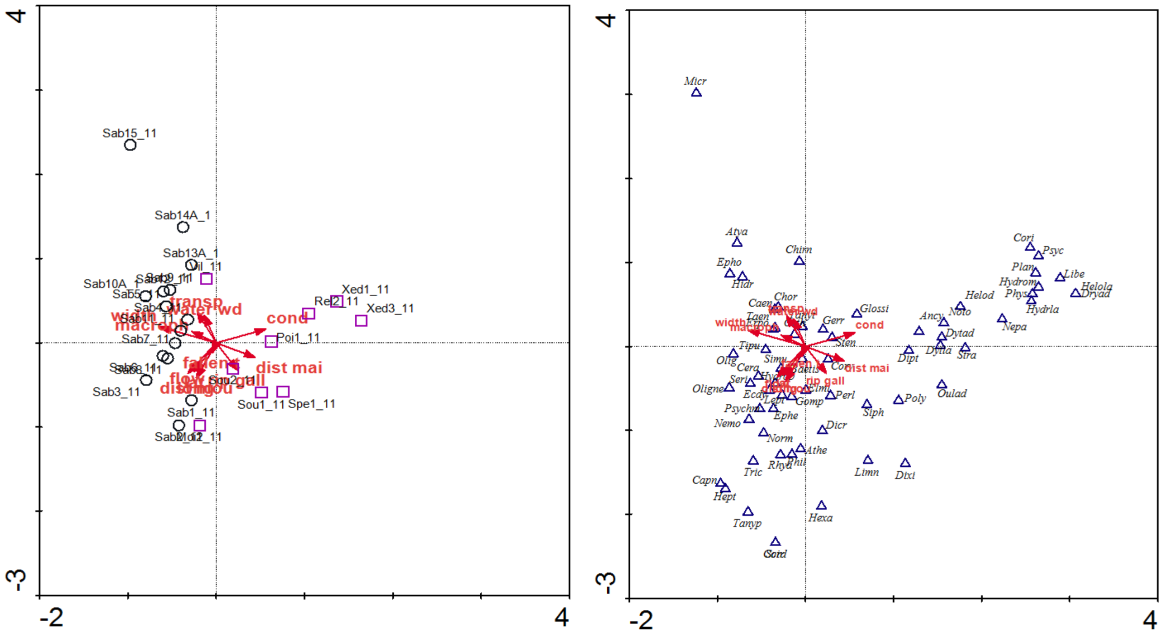


Fig. 11. CCA biplots for 2011 data. Circles (main course of the river) and squares (tributaries) stand for sampling sites, whilst triangles represent species (for taxa abbreviations, see Table S1). Arrows represent explanatory variables in both diagrams.

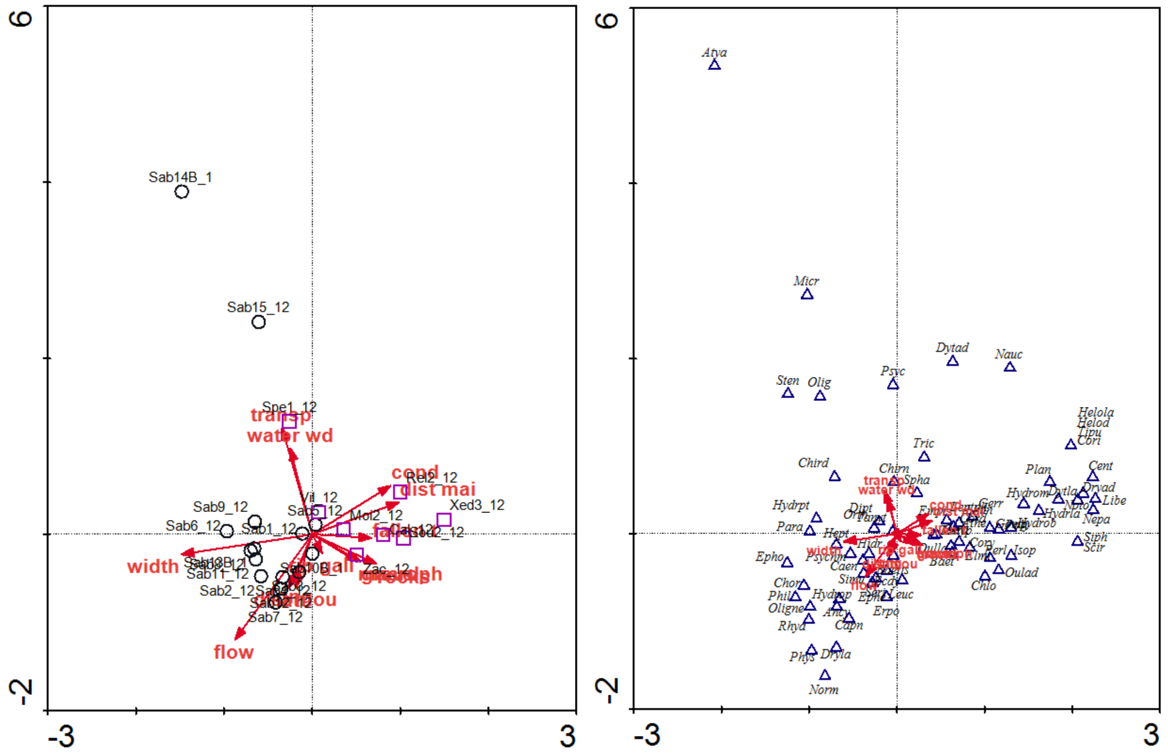


Fig.12. CCA biplots for 2012 data. Circles (main course of the river) and squares (tributaries) stand for sampling sites, whilst triangles represent species (for taxa abbreviations, see Table S1). Arrows represent explanatory variables in both diagrams.

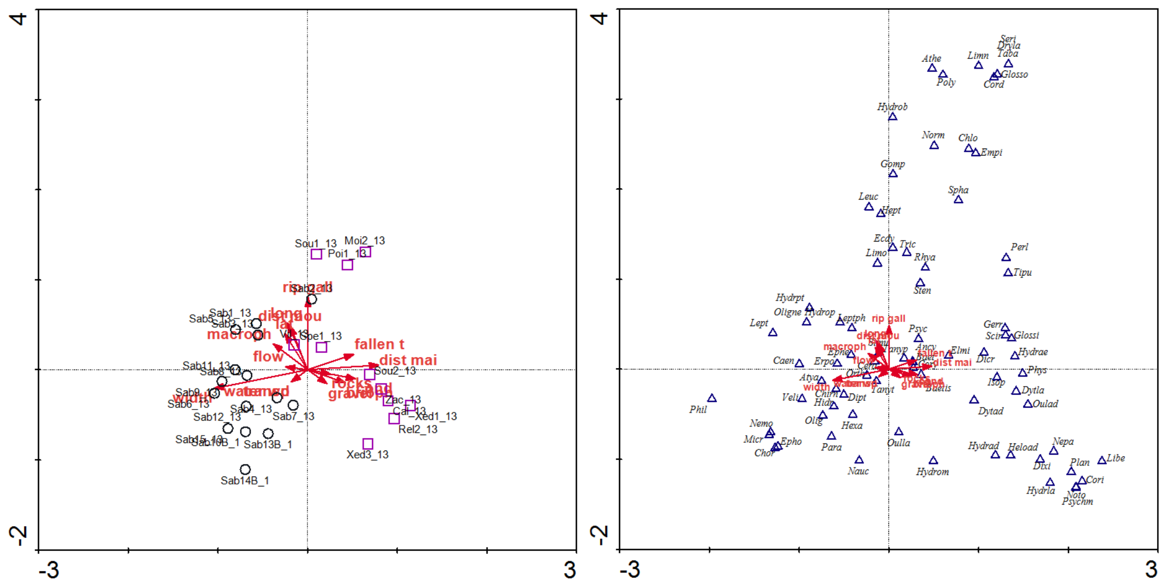


Fig. 13. CCA biplots for 2013 data. Circles (main course of the river) and squares (tributaries) stand for sampling sites, whilst triangles represent species (for taxa abbreviations, see Table S1). Arrows represent explanatory variables in both diagrams.

The beginning of the construction of the dams (june 2008) seems to have affected negatively the ecological status of the river, although limited locally and temporally. The results showed that tributaries, more diversified and rich, are the most important contributors of biodiversity in the hydrographic basin. Peralta (2004) states that a high diversity it is, generally, characteristic of stable communities and that occupy favourable and varied habitats. On the contrary, a low diversity is usually associated with unstable communities or exposed to unfavourable fluctuations of the environmental conditions. In fact, the richness of the Sabor river Valley stems from its heterogeneous landscape. Unfortunately, this heterogeneity will be destroyed by the filling of the reservoir (from 2014 onwards). Also, many of the important tributaries studied here will be flooded, which will seriously impair their biodiversity value. As to the ongoing construction, the data collected during 2009-2013 did not detect important changes in the invertebrate community, which mostly responded to environmental inter-annuum shifts. However, the present evidences for poor/moderate ecological status in some sites of the river should alert stakeholders for future basin (and reservoir) management.

References

Abràmoff, M. D., Magalhães, P.J., & Ram, S.J. (2004). Image processing with imageJ. Biophotonics International, 11, 36–41.

Alba-Tercedor, J., & Sánchez-Ortega, A. (1988). Un método rápido y simple para evaluar la calidad biológica de las águas corrientes basado en el de Hellawell (1978). Limnética, 4, 51–56.

Ancog, R., Andrade, G.B., Miasco, R.B., & Ortiz, M.F. (2010). Water quality and diversity of macroinvertebrate species in rivers of Cebu City, Philippines. The Philippine Scientist, 47, 27-45.

Aschonitis, V.G., Feld, C.K, Castaldelli, G., Turin, P., Visonà, E. & Fano, E.A. (2016). Environmental stressor gradients hierarchically regulate macrozoobenthic community turnover in lotic systems of Northern Italy. Hydrobiologia, 765, 131–147.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society. Series B (Methodological), 57, 289–300.

Blijswijk, W.V., Coimbra, C.N., & Graça, M.A.S. (2004). The use of biological methods based on macroinvertebrates to an Iberian stream (Central Portugal) receiving a paper mill effluent. Limnetica, 23, 307–314.

Braak, C. ter, & Smilauer, P. (1998). CANOCO reference manual and user's guide to Canoco for Windows. Software for Canonical Community Ordination (version 4). Centre for Biometry, Wageningen.

Castro, B.B., Antunes, S.C., Pereira, R., Soares, A.M.V.M., & Gonçalves, F. (2005). Rotifer community structure in three shallow lakes: seasonal fluctuations and explanatory factors. Hydrobiologia, 543, 221–232.

Chapman, D., & Jackson, J. 81996). Biological Monitoring In Bartram, J., & R. Ballance (eds), Water Quality Monitoring - A Practical Guide to the Design and Implementation of Freshwater Quality Studies and Monitoring Programmes. United Nations Environment Programme and the World Health Organization, 263–302.

Davy-Bowker, J., & Furse, M. (2006). Hydromorphology — major results and conclusions from the STAR project. The Ecological Status of European Rivers: Evaluation and Intercalibration of Assessment Methods SE. Developments in Hydrobiology, 188, 263–265.

Dunbar, M. J., Pedersen, M.L., Cadman, D., Extence, C., Waddingham, J., Chadd, R., & Larsen, S.E. (2010a). River discharge and local-scale physical habitat influence macroinvertebrate LIFE scores. Freshwater Biology, 55, 226–242.

Dunbar, M. J., Warren, M., Extence, C., Baker, L., Cadman, D., Mould, D.J., Hall, J., & Chadd, R. (2010b). Interaction between macroinvertebrates, discharge and physical habitat in upland rivers. Aquatic Conservation: Marine and Freshwater Ecosystems, 20, 31–44.

Edington, J.M., & Hildrew, A.G. (2005). A Revised Key to the Caseless Caddis Larvae of the British Isles. Freshwater Biological Association.

EEA (2015). The European environment — state and outlook 2015: synthesis. Copenhagen, Denmark.

Elliott, J.M., & Humpesch, U.H. (2010). Mayfly Larvae (Ephemeroptera) of Britain and Ireland: Keys and a Review of their Ecology. Ambleside:The Freshwater Biological Association.

Extence, C. A., Chadd, R.P.,. England, J, Dunbar, M.J., Wood, P.J., & Taylor, E.D.(2013). The assessment of fine sediment accumulation in rivers using macro-invertebrate community response. River Research and Applications, 29, 17–55.

Freitas, H., & Horta, K. (2003). Proposed Portugal Dam Would Flood Culturally Rich, Biologically Diverse Area. World Rivers Review, 18, 10–11.

García-Ruiz, J.M., López-Moreno, J.I., Vicente-Serrano, S.M., Lasanta–Martínez, T., & Beguería, S. (2011). Mediterranean water resources in a global change scenario. Earth-Science Reviews Elsevier B.V., 105, 121–139.

Grill, G., Dallaire, C.O., Chouinard, E.F., Sindorf, N., & Lehner, B. (2014). Development of new indicators to evaluate river fragmentation and flow regulation at large scales : A case study for the Mekong River Basin. Ecological Indicators Elsevier, 45, 148–159.

Heathwaite, A.L., (2010). Multiple stressors on water availability at global to catchment scales: understanding human impact on nutrient cycles to protect water quality and water availability in the long term. Freshwater Biology, 55, 241–257.

Hellawell, J.M. (1986). Biological indicators of freshwater pollution and environmental management. Elsevier Applied Science Publishers.

Hering, D., Bufagni, A., Moog, O., Sandin, L., Sommerhäuser, M., Stubauer, I., … Zahrádková, S. (2003). The development of a system to assess the ecological quality of streams based on macroinvertebrates – design of the sampling programme within the AQEM project. International Review of Hydrobiology, 88: 345–361.

Hering, D., Johnson, R.K., Kramm, S., Schmutz, S., Szoszkiewicz, K., & Verdonschot, P.F.M. (2006). Assessment of European streams with diatoms, macrophytes, macroinvertebrates and fish: A comparative metric-based analysis of organism response to stress. Freshwater Biology, 51, 1757–1785.

Hoelzer, A. (2003). Vegetation ecological studies at the lower course of Sabor River (Tras-os-Montes, NE-Portugal). University Bremen, http://www.grin.com/en/e-book/28444/vegetation-ecological-studies-at-the-lower-course-of-sabor-river-tras-os-montes.

Hynes, H.B.N. (1993). A Key to the Adults and Nymphs of the British Stoneflies (Plecoptera): With Notes on Their Ecology and Distribution. Freshwater Biological Association.

INAG (2008a). Manual para a avaliação biológica da qualidade da água em sistemas fluviais segundo a Directiva Quadro da Água - Protocolo de amostragem e análise para os macroinvertebrados bentónicos. Lisboa: Ministério do Ambiente, Ordenamento do Território e do Desenvolvimento Regional.

INAG (2008b). Tipologia de rios em Portugal Continental no âmbito da implementação da Directiva Quadro da Água, I - Caracterização abiótica. Lisboa: Ministério do Ambiente, Ordenamento do Território e do Desenvolvimento Regional.

INAG (2009). Critérios para a Classificação do Estado das Massas de Água Superficiais- Rios e Albufeiras. Lisboa: Ministério do Ambiente, Ordenamento do Território e do Desenvolvimento Regional.

INE (2012). Censos 2011 Resultados Definitivos - Região Norte. Lisboa, Portugal.

IPCC (2014). Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge: Cambridge University Press.

Jackson, A.L.R. (2011). Renewable energy vs. biodiversity: Policy conflicts and the future of nature conservation. Global Environmental Change Elsevier, 21, 1195–1208.

JRC, 2009. Water Framework Directive intercalibration technical report - Part 1: Rivers.

Looy, K.V., Tormos, T., & Souchon, Y. (2014). Disentangling dam impacts in river networks. Ecological Indicators, 37, 10-20.

Lorenz, A., Hering, D., Feld, C.K, & Rolauffs, P. (2004). A new method for assessing the impact of hydromorphological degradation on the macroinvertebrate fauna of five German stream types. Hydrobiologia, 516, 107–127.

Melo, J.J. De (2009). Public works policy in Portugal : a case study in unsustainability João Joanaz de Melo. International Journal of Engineering and Industrial Management, 1, 195–208.

Metcalfe-Smith, J.L. (1994). Biological Water-Quality Assessment of Rivers: Use of Macroinvertebrate Communities. In P. Calow & G. E., Petts (Eds.), The Rivers Handbook: Hydrological and Ecological Principles. Oxford: Blackwell Science Ltd.

Munné, A., & Prat, N. (2009). Use of macroinvertebrate-based multimetric indices for water quality evaluation in Spanish Mediterranean rivers: An intercalibration approach with the IBMWP index. Hydrobiologia, 628, 203–225.

Paterson, J. S., Araújo, M.B., Berry, P.M., Piper, J.M., & Rounsevell, M.D. (2008). Mitigation, adaptation, and the threat to biodiversity. Conservation Biology, 22, 1352–1355.

Pawley, S., Dobson, M., & Fletcher, M. (2011). Guide to British Freshwater Macroinvertebrates for Biotic Assessment. Ambleside: The Freshwater Biological Association.

Peralta, N.R.E. (2004). Levantamento de Invertebrados Bentónicos na Zona de Protecção Especial do Paul do Taipal. Instituto de Conservação da Natureza - Reserva Natural do Paul de Arzila.

Poff, N.L., Pyne, M.I., Bledsoe, B.P., Cuhaciyan, C.C., & Carlisle, D.M. (2010). Developing linkages between species traits and multiscaled environmental variation to explore vulnerability of stream benthic communities to climate change. Journal of the North American Benthological Society, 29, 1441–1458.

QGIS Development Team (2015). QGIS Geographic Information System. Open Source Geospatial Foundation Project. Open Source Geospatial Foundation Project, http://www.qgis.org/es/site/.

R Core Development Team (2015). R: a language and environment for statistical computing, 3.2.1. Document freely available on the internet at: http://www. r-project. org. R Foundation for Statistical Computing, Vienna, Austria, www.R-project.org.

Revelle, W. (2016). Psych: Procedures for personality and psychological research. Northwestern university. Northwestern University, Evanston, Illinois, USA, https://scholar.google.de/scholar?q=Procedures+for+Personality+and+Psychological+Research+revelle&btnG=&hl=de&as_sdt=0%2C5#0.

Serra, S., Coimbra, N., & Graça, M. (2009). Invertebrados de Água Doce: Chave de Identificação Das Principais Famílias. Coimbra: Imprensa da Universidade de Coimbra.

Sundermann, A., Lohse, S., Beck, L.A., Haase, P. (2007). Key to the larval stages of aquatic true flies (Diptera), based on the operational taxa list for running waters in Germany. Annales De Limnologie-International Journal of Limnology, 43, 61-74.

Tachet, H. (2000). Invertébrés d'eau douce : Systématique, biologie, écologie. CNRS Editions.

ter Braak, C.J.F. (1995). Ordination In R. H. G. Jongman, C. J. F. ter Braak, & O. F. R. Tongeren (Eds.), Data Analysis in Community and Landscape Ecology. Cambridge: Cambridge University Press.

Varol, M.B., Gökot, A., Bekleyen, B., & Şen, B. (2012). Spatial and temporal variations in surface water quality of the dam reservoirs in the Tigris River basin, Turkey. Catena, 92, 11–21.

Vidal, T., Santos, J.I., Marques, C.R., Pereira, J.L., Claro, M.T., Pereira, R., Castro, B.B., … Gonçalves (2014). Resilience of the macroinvertebrate community of a small mountain river (Mau River , Portugal) subject to multiple stresses. Marine and Freshwater Research, 65, 633–644.

Wallace, I.D., Wallace, B., & Philipson, G.N. (2003). Keys to the Case-bearing Caddis Larvae of Britain and Ireland. Ambleside: The Freshwater Biological Association

Xiaoyan, L., Shikui, D., Qinghe, Z., & Shiliang, L. (2010). Impacts of Manwan Dam construction on aquatic habitat and community in Middle Reach of Lancang River. Procedia Environmental Sciences, 706–712.

Zganec, K., Đuric, P., Hudina, S., & Gottstein, S. (2013). Population and distribution changes of two coexisting river amphipods after the closure of a new large dam. Limnologica, 43, 460–468.

Supplementary Material

Table S1. Macroinvertebrate abundance in each sampling site in 2009.

	Abbrev	Sab7_09	Sab8_09	Sab9_09	Sab10A_09	Sab12_09	Sab13A_09	Sab14A_09	Xed1_09	Xed2_09	Xed3_09	Rel1_09	Rel2_09	Moi1_09	Moi2_09	Spe1_09	Spe2_09	Spe3_09	Sou1_09	Sou2_09	Jun1_09	Jun2_09	Jun3_09	Poi1_09	Poi2_09	Mac1_09	Mac2_09
Ancylus	Ancy	0	0	8	0	1	1	0	10	22	0	1	1	11	3	0	0	4	1	1	3	3	2	0	2	0	1
Athericidae	Athe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	1	0	0	0
Atyaephyra	Atya	0	2	2	0	81	1	6	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
Baetidae (others)	Baet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Baetis	Baetis	4	1	840	0	47	61	1	0	38	1	14	43	97	3	3	6	57	43	30	49	6	75	0	0	1	0
Caenis	Caen	34	103	45	0	51	44	0	0	5	2	0	15	0	0	0	14	11	0	0	0	0	0	0	0	54	70
Capniidae	Capn	0	0	0	0	0	0	0	0	0	2	0	2	38	0	0	0	0	0	115	0	9	0	0	9	0	0
Centroptilum	Cent	0	0	0	0	0	0	0	0	0	1	260	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ceratopogonidae	Cera	0	0	0	0	0	0	0	0	0	0	0	0	7	0	34	0	0	0	0	2	2	2	0	0	1	0
Chironomidae (others)	Chird	0	0	0	0	0	0	0	0	1	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Chironomini	Chirn	1	7	0	18	0	0	2	0	0	1	3	0	148	177	1	2	0	0	0	0	8	3	0	0	6	11
Chloroperlidae	Chlo	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0	2	0	0	0	0	0
Choroterpes	Chor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cordulegasteridae	Cord	0	0	0	0	0	1	0	0	0	0	0	0	6	1	0	0	0	0	1	0	0	0	0	0	0	0
Corixidae (others)	Cori	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corynoneura	Cory	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dicranota	Dicr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diptera n.i.	Dipt	19	1	168	2	14	30	0	0	3	1	8	2	4	6	4	0	0	0	0	1	3	3	9	1	7	1
Dixidae	Dixi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Dryops (adult)	Dryad	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0
Dryops (larva)	Dryla	0	0	5	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0
Dupophilus (adult)	Dupo	0	0	3	0	2	2	0	0	0	1	0	0	10	0	1	1	29	0	2	4	2	3	0	0	0	0
Dytiscidae (adult)	Dytad	0	0	0	0	9	0	0	0	0	13	10	9	0	0	0	1	2	0	0	17	9	13	7	1	0	0
Dytiscidae (larva)	Dytla	0	0	0	0	0	0	0	0	4	7	55	23	0	0	7	2	3	1	1	6	2	2	2	4	0	0
Ecdyonurus	Ecdy	0	0	0	0	3	0	0	0	0	0	0	0	5	1	0	0	0	0	19	0	0	1	0	1	0	0
Elmidae (others)	Elmi	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	4	0	0	0	0	0	0	0	0
Empididae	Empi	0	0	0	0	0	0	0	0	0	0	0	0	38	0	0	0	0	0	0	0	0	0	0	0	0	0
Ephemerella	Ephe	15	1	1312	1	26	15	0	0	0	0	1	0	3	0	0	4	28	3	2	3	5	28	1	0	0	0
Ephoron	Epho	0	0	213	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Erpobdellidae	Erpo	0	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Gerridae	Gerr	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	6	0	0	0	0	0	0	0
Glossiphoniidae	Glossi	0	0	0	0	0	0	0	1	1	2	0	0	0	0	0	1	0	0	0	3	0	0	0	0	0	0
Glossosomatidae	Glosso	0	0	0	0	0	0	0	0	0	0	0	0	71	65	1	24	3	10	2	0	0	2	0	6	0	0
Gomphidae	Gomp	0	1	6	1	0	0	0	0	0	0	0	0	5	0	0	0	0	0	2	0	0	0	0	0	0	0
Helodidae (larva)	Helod	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	7	0	3	0	0	0	0
Helophorus (adult)	Heload	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helophorus (larva)	Helola	0	0	0	0	0	0	0	0	0	0	2	0	11	0	0	0	0	0	0	1	0	0	0	0	0	0
Heptageniidae (others)	Hept	0	0	3	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hexatoma	Hexa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hidracarina	Hidr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	0	1	0
Hydraenidae (adult)	Hydrae	0	0	0	0	0	0	0	0	0	0	0	0	31	0	10	0	0	8	1	1	0	2	0	0	0	0
Hydrobiidae	Hydrob	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	246	0	0	0	0	0	0	0
Hydrometridae	Hydrom	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrophilidae (adult)	Hydrad	0	0	0	0	0	0	0	0	0	0	2	1	0	0	4	0	0	0	0	1	0	0	0	0	0	0
Hydrophilidae (larva)	Hydrla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydropsychidae	Hydrop	4	0	104	0	0	5	0	0	0	0	0	0	39	5	1	1	0	0	4	0	0	0	0	0	0	0
Hydroptilidae	Hydrpt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Isoperla	Isop	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptoceridae	Lept	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	8	6	4	0	0	0	0
Leptophlebiidae (others)	Leptph	0	1	3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	3	0
Leuctridae	Leuc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Libellulidae	Libe	0	0	0	0	0	0	0	0	9	0	4	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Limnephilinae	Limn	0	0	0	0	0	0	0	0	0	0	0	0	123	1	1	1	3	0	2	1	1	1	1	0	0	0
Limoniidae (others)	Limo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	2	0	0	0	0	0
Micronecta	Micr	0	116	0	0	15	0	182	0	1	0	5	39	0	0	0	0	0	0	0	0	0	0	0	0	568	0
Naucoridae	Nauc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nemouridae	Nemo	0	0	21	1	0	0	0	0	7	0	5	3	193	0	16	0	3	89	12	69	21	44	0	3	0	0
Nepa	Nepa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Normandia (larva)	Norm	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	4	0	0	0	0	0	0	0
Notonectidae	Noto	0	0	0	0	0	0	0	0	0	0	0	8	0	0	1	8	1	0	0	0	0	0	0	0	0	0
Oligochaeta	Olig	0	0	0	4	0	0	0	0	17	0	0	0	2	0	3	0	1	0	0	3	8	0	0	0	0	0
Oligoneuriidae	Oligne	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Orthocladiinae	Orth	296	9	1616	12	32	48	0	0	1	1	4	0	200	178	8	2	12	1	2	10	4	12	20	4	2	0
Oulimnius (adult)	Oulad	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oulimnius (larva)	Oulla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paraleptophlebia	Para	0	0	0	0	0	0	0	0	0	0	0	0	143	0	0	0	0	0	2	0	0	0	0	0	0	0
Perlodidae (others)	Perl	0	0	2	0	0	0	0	0	1	1	0	1	32	1	7	129	62	73	5	103	16	138	3	5	0	0
Philopotamidae	Phil	0	0	0	0	0	0	0	0	0	0	0	0	4	1	0	1	0	0	0	3	1	0	0	0	0	0
Physidae	Phys	0	0	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Planorbis	Plan	0	0	0	0	0	0	0	0	0	1	31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Polycentropodidae	Poly	0	0	0	0	0	0	0	0	0	0	0	0	6	1	0	0	1	2	3	0	0	0	0	0	0	0
Psychodidae	Psyc	0	0	0	0	0	0	0	0	0	0	0	0	5	0	1	0	0	0	0	0	0	7	0	0	0	0
Psychomyiidae	Psychm	0	0	0	0	0	0	0	0	1	0	0	0	3	0	0	0	1	0	1	0	0	0	0	0	0	0
Rhyacophila	Rhya	0	0	27	0	0	1	0	0	0	0	0	0	7	0	0	0	4	0	1	1	0	1	0	0	0	0
Scirtidae	Scir	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sericostomatidae	Seri	0	0	3	0	1	2	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0
Simuliidae	Simu	93	0	3360	0	43	50	0	0	162	0	0	0	4	0	1	0	2	0	0	1	0	0	0	0	0	0
Siphlonurus	Siph	0	0	2	0	0	0	0	0	59	0	0	0	0	0	5	138	52	4	0	1	17	7	2	45	0	0
Sphaeridae	Spha	0	0	2	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Stenelmis (larva)	Sten	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stratiomyidae	Stra	0	0	0	0	0	0	0	0	1	1	0	0	0	0	5	0	0	0	0	10	2	6	0	0	0	0
Tabanidae	Taba	0	0	0	0	0	0	0	0	1	0	0	0	0	0	4	0	0	0	0	0	0	0	0	1	0	0
Taeniopterygidae	Taen	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	0	1	0	0	0	0
Tanypodinae	Tanyp	0	0	0	0	0	0	0	0	5	2	11	3	269	26	0	0	0	0	0	6	82	24	0	0	0	0
Tanytarsini	Tanyt	0	0	0	0	3	0	1	0	0	21	1	0	349	10	1	0	0	0	0	2	1	2	1	5	6	0
Tipulidae	Tipu	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Trichoptera (others)	Tric	1	0	6	0	0	0	0	0	2	0	0	0	19	16	0	1	0	0	0	0	0	0	0	0	0	0
Veliidae	Veli	0	0	0	0	0	0	0	0	1	0	1	5	0	0	2	0	0	0	0	0	1	3	0	0	0	0

Table S2. Macroinvertebrate abundance in each sampling site in 2010.

	Abbrev	Sab1_10	Sab2_10	Sab3_10	Sab4_10	Sab5_10	Sab6_10	Sab7_10	Sab8_10	Sab9_10	Sab10A_10	Sab11_10	Sab12_10	Sab13A_10	Sab14A_10	Sab15_10	Xed1_10	Xed2_10	Xed3_10	Rel1_10	Rel2_10	Moi1_10	Moi2_10	Spe1_10	Spe2_10	Spe3_10	Sou1_10	Sou2_10	Jun1_10	Jun2_10	Jun3_10	Poi1_10	Poi2_10	Mac1_10	Mac2_10
Ancylus	Ancy	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	4	2	4	1	0	1	0	1	6	8	0	8	2	5	1	1	0	0
Athericidae	Athe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	6	0	17	0	0	0	0	0	0	0	0	0	0	0	0
Atyaephyra	Atya	0	0	0	0	4	0	0	0	0	3	0	0	0	69	79	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13
Baetidae (others)	Baet	0	0	0	0	0	0	0	0	0	0	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Baetis	Baetis	13	0	1	8	0	14	0	1	1	0	0	101	5	0	21	0	62	77	27	78	360	222	0	183	126	0	8	1	0	7	0	10	5	0
Caenis	Caen	6	6	12	10	0	4	8	3	1	0	0	0	26	0	4	0	0	0	0	1	0	0	0	0	31	0	0	0	0	0	0	0	4	7
Capniidae	Capn	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	3	20	4	0	0	0	0	0	0	2	0	0	7	0	2	4	0	0
Centroptilum	Cent	0	0	2	25	5	0	5	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Ceratopogonidae	Cera	1	0	1	1	0	0	0	0	1	0	0	3	0	0	0	0	0	0	4	0	2	3	0	6	2	2	0	0	1	0	0	1	0	0
Chironomidae (others)	Chird	0	0	0	0	0	0	0	0	0	0	63	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chironomini	Chirn	0	0	0	2	0	0	0	3	0	0	0	0	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chloroperlidae	Chlo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Choroterpes	Chor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cordulegasteridae	Cord	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	9	0	0	1	0	0	0	0	0	0	0	0	0
Corixidae (others)	Cori	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corynoneura	Cory	0	2	1	3	0	0	0	2	1	3	0	21	8	0	2	0	10	1	7	79	1	1	32	0	0	0	0	4	4	0	95	0	0	0
Dicranota	Dicr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	3	10	0	0	0	0
Diptera n.i.	Dipt	1	7	13	7	2	2	8	1	9	0	4	5	3	0	0	0	22	16	4	3	5	8	4	3	6	7	7	2	4	0	15	0	11	1
Dixidae	Dixi	0	0	0	0	0	0	0	0	0	0	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Dryops (adult)	Dryad	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	7	0	0	0
Dryops (larva)	Dryla	0	0	3	0	6	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Dupophilus (adult)	Dupo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Dytiscidae (adult)	Dytad	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	7	0	0	0	0	1	0	0	0	0	0	1	2	0	0
Dytiscidae (larva)	Dytla	0	0	0	0	0	1	0	0	0	0	0	5	0	0	0	0	0	0	0	2	0	0	0	1	2	1	1	0	0	0	2	4	0	0
Ecdyonurus	Ecdy	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	40	20	0	1	2	0	17	0	0	0	0	1	0	0
Elmidae (others)	Elmi	1	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Empididae	Empi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	2	0	0	0
Ephemerella	Ephe	0	0	0	0	0	0	2	0	0	0	0	0	0	0	3	0	0	0	1	0	0	0	0	33	7	0	2	0	0	0	0	2	0	3
Ephoron	Epho	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Erpobdellidae	Erpo	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gerridae	Gerr	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glossiphoniidae	Glossi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Glossosomatidae	Glosso	0	0	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	39	14	1	65	0	10	2	3	0	1	0	3	0	0
Gomphidae	Gomp	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	21	0	0	1	0	2	0	0	0	0	0	0	0
Helodidae (larva)	Helod	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	193	1	1	0	0	19	0	0	0	1	0	0	0	0
Helophorus (adult)	Heload	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helophorus (larva)	Helola	0	2	0	2	0	0	1	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heptageniidae (others)	Hept	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hexatoma	Hexa	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	3	2	2	0	0	0	0	10	0	0	1	1	21	36	0	2	0	0	0
Hidracarina	Hidr	0	0	0	0	3	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
Hydraenidae (adult)	Hydrae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	3	0	0	0	0	5	0	0	0
Hydrobiidae	Hydrob	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0
Hydrometridae	Hydrom	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrophilidae (adult)	Hydrad	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	6	0	0	0
Hydrophilidae (larva)	Hydrla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydropsychidae	Hydrop	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	14	4	0	0	0	0	0	0	0	0	0	0	0
Hydroptilidae	Hydrpt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Isoperla	Isop	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptoceridae	Lept	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptophlebiidae (others)	Leptph	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0
Leuctridae	Leuc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Libellulidae	Libe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Limnephilinae	Limn	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	2	3	10	0	1	0	0	3	2	6	6	2	0	4	1
Limoniidae (others)	Limo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Micronecta	Micr	0	0	0	0	0	1	0	16	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Naucoridae	Nauc	35	1	0	0	0	0	0	0	0	0	0	0	0	16	191	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nemouridae	Nemo	1	1	0	6	0	19	0	0	0	0	0	0	0	0	0	81	76	103	48	48	0	0	38	0	0	73	7	113	42	0	112	64	17	0
Nepa	Nepa	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Normandia (larva)	Norm	0	1	1	0	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Notonectidae	Noto	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oligochaeta	Olig	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	3	4	0	0	0	0	2	0	0	0	0	1	0	0
Oligoneuriidae	Oligne	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Orthocladiinae	Orth	20	52	168	147	35	117	69	21	53	22	0	96	232	0	13	85	148	145	140	344	369	156	212	203	149	65	242	81	73	14	341	15	180	156
Oulimnius (adult)	Oulad	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	343	66	2	0	0	0	2	0	0	0	0
Oulimnius (larva)	Oulla	0	0	0	0	0	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paraleptophlebia	Para	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	269	172	2	0	0	0	3	0	0	0	0	0	0	0
Perlodidae (others)	Perl	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	39	40	49	11	17	13	0	3	133	104	55	5	27	10	38	19	31	2	0
Philopotamidae	Phil	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0	0	0	0	2	0	0	0	5	0	0
Physidae	Phys	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0
Planorbis	Plan	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	7	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Polycentropodidae	Poly	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Psychodidae	Psyc	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
Psychomyiidae	Psychm	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhyacophila	Rhya	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Scirtidae	Scir	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sericostomatidae	Seri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	19	0	0	0	0	0	0	0	0	0	0	0	0
Simuliidae	Simu	0	4	20	12	0	6	26	0	2	0	30	272	13	0	0	45	58	164	0	155	0	0	209	3	0	24	7	9	87	0	38	64	3	2
Siphlonurus	Siph	0	7	15	8	0	4	1	0	2	0	0	0	3	1	0	0	0	0	0	0	0	0	0	33	0	0	0	0	0	0	0	0	0	2
Sphaeridae	Spha	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0
Stenelmis (larva)	Sten	0	1	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stratiomyidae	Stra	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	3	0	1	0	0	2	2	2	0	0	0
Tabanidae	Taba	0	0	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	2	0	0	0	0	0
Taeniopterygidae	Taen	0	0	0	0	0	2	2	0	0	0	0	0	5	0	0	28	26	8	2	3	0	0	6	0	0	13	5	28	34	6	1	14	1	0
Tanypodinae	Tanyp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tanytarsini	Tanyt	1	3	16	2	1	1	0	1	6	2	0	8	2	0	5	7	11	7	5	13	77	97	0	6	0	0	0	0	2	0	25	0	0	1
Tipulidae	Tipu	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	2	3	0	1	0	1	1	0	2	1	0	0	0
Trichoptera (others)	Tric	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Veliidae	Veli	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S3. Macroinvertebrate abundance in each sampling site in 2011.

	Abbrev	Sab1_11	Sab2_11	Sab3_11	Sab4_11	Sab5_11	Sab6_11	Sab7_11	Sab8_11	Sab9_11	Sab10A_11	Sab11_11	Sab12_11	Sab13A_11	Sab14A_11	Sab15_11	Xed1_11	Xed3_11	Rel2_11	Moi2_11	Spe1_11	Sou1_11	Sou2_11	Poi1_11	Vil_11
Ancylus	Ancy	0	6	0	0	0	0	0	0	0	8	1	4	0	0	0	48	415	82	0	6	10	11	5	0
Athericidae	Athe	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	4	0	0
Atyaephyra	Atya	3	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1
Baetidae (others)	Baet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Baetis	Baetis	283	2865	1146	848	13	185	2089	130	677	24	196	431	2	2	0	101	383	2232	840	49	1360	460	166	7
Caenis	Caen	467	25	25	75	38	89	0	48	463	16	40	64	6	58	13	1	14	181	32	12	0	29	5	43
Capniidae	Capn	0	0	594	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	192	0	0	0	0	0
Centroptilum	Cent	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ceratopogonidae	Cera	63	29	45	19	4	9	4	0	21	8	1	3	1	3	0	0	0	0	20	21	0	58	2	0
Chironomidae (others)	Chird	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chironomini	Chirn	0	0	8	0	96	122	8	0	0	320	0	0	56	0	7	56	112	0	0	10	0	0	0	177
Chloroperlidae	Chlo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Choroterpes	Chor	0	16	98	455	17	129	49	146	1722	0	21	11	3	121	36	2	45	90	496	7	0	510	4	34
Cordulegasteridae	Cord	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	0	0	0
Corixidae (others)	Cori	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0
Corynoneura	Cory	0	1	3	52	1	0	0	0	0	0	0	0	0	0	0	0	13	0	4	0	0	0	2	0
Dicranota	Dicr	0	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	7	5	0
Diptera n.i.	Dipt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	11	16	13	32	4	10	4	39
Dixidae	Dixi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	12	0	0	0
Dryops (adult)	Dryad	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
Dryops (larva)	Dryla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dupophilus (adult)	Dupo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dytiscidae (adult)	Dytad	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	51	103	89	0	23	24	40	36	1
Dytiscidae (larva)	Dytla	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	58	96	80	0	94	87	12	44	0
Ecdyonurus	Ecdy	0	88	92	16	0	30	190	13	11	0	21	2	0	0	1	0	0	0	33	31	4	271	21	0
Elmidae (others)	Elmi	0	7	16	24	0	0	0	0	0	0	0	0	0	0	2	0	10	0	16	0	11	20	0	0
Empididae	Empi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ephemerella	Ephe	254	177	66	45	3	71	90	4	9	0	4	0	0	0	0	0	0	1	12	83	12	9	10	0
Ephoron	Epho	0	0	0	81	43	280	311	261	446	0	21	44	0	40	4	0	0	0	0	1	0	0	2	2
Erpobdellidae	Erpo	0	0	0	0	0	0	8	17	0	0	8	0	0	0	1	0	1	0	1	0	0	0	4	0
Gerridae	Gerr	32	0	1	0	3	12	8	0	0	0	3	0	3	6	4	0	23	6	11	36	0	21	31	7
Glossiphoniidae	Glossi	0	0	0	0	0	0	0	0	0	0	26	0	0	0	0	0	4	0	0	0	0	0	1	0
Glossosomatidae	Glosso	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gomphidae	Gomp	1	1	1	1	0	0	0	2	11	0	0	0	0	0	0	0	0	4	59	1	0	10	0	1
Helodidae (larva)	Helod	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	280	4	0	0	0	0	30	0	0
Helophorus (adult)	Heload	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helophorus (larva)	Helola	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	34	0	0	0	0	0	0	0
Heptageniidae (others)	Hept	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Hexatoma	Hexa	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	15	0	0	0
Hidracarina	Hidr	41	1	3	495	43	117	8	58	4	0	5	47	8	176	11	0	1	1	0	1	0	6	0	4
Hydraenidae (adult)	Hydrae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrobiidae	Hydrob	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrometridae	Hydrom	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	5	0	0	0	0	0	0
Hydrophilidae (adult)	Hydrad	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrophilidae (larva)	Hydrla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	48	1	0	0	0	0	2	0
Hydropsychidae	Hydrop	737	1611	1005	104	0	27	2196	748	174	0	155	124	0	0	0	6	2	49	35	80	25	25	1	0
Hydroptilidae	Hydrpt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Isoperla	Isop	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptoceridae	Lept	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0
Leptophlebiidae (others)	Leptph	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leuctridae	Leuc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Libellulidae	Libe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	32	0	0	0	0	0	0	0
Limnephilinae	Limn	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	12	0	5	0	0
Limoniidae (others)	Limo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Micronecta	Micr	0	0	0	0	0	0	0	0	40	56	1	0	32	5	656	0	0	0	0	0	0	0	0	0
Naucoridae	Nauc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nemouridae	Nemo	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0
Nepa	Nepa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	12	8	0	1	2	0	0	0
Normandia (larva)	Norm	8	0	30	0	0	6	24	7	0	0	0	0	0	0	0	0	0	0	27	0	16	25	0	0
Notonectidae	Noto	8	0	0	0	0	0	0	0	7	0	0	0	0	0	0	39	88	128	0	6	2	0	27	0
Oligochaeta	Olig	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Oligoneuriidae	Oligne	3	220	111	14	0	3	157	25	0	0	27	8	0	0	0	1	0	0	0	0	0	0	0	0
Orthocladiinae	Orth	465	2317	233	353	22	101	1423	116	353	128	353	166	7	23	13	183	524	164	128	163	86	249	24	59
Oulimnius (adult)	Oulad	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	96	0	32	4	425	36	0	0	0
Oulimnius (larva)	Oulla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paraleptophlebia	Para	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Perlodidae (others)	Perl	312	0	1	13	0	25	2	0	7	0	0	0	0	0	0	3	0	54	5	48	170	7	0	0
Philopotamidae	Phil	16	135	2	0	0	1	0	11	0	0	0	0	0	0	0	0	13	0	8	0	0	1	0	0
Physidae	Phys	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1939	104	0	1	0	0	0	0
Planorbis	Plan	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1390	2986	123	0	0	0	0	0	0
Polycentropodidae	Poly	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	4	12	1	0	35	0	0
Psychodidae	Psyc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	1	0	0	0	0	0	0	0
Psychomyiidae	Psychm	8	0	1	0	0	0	0	0	0	0	0	12	0	0	0	0	0	0	8	0	0	7	0	0
Rhyacophila	Rhya	0	100	15	0	0	0	3	0	0	0	0	0	0	0	0	0	2	0	0	3	0	1	0	0
Scirtidae	Scir	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	299	0	0	0	0	0
Sericostomatidae	Seri	0	0	1	0	0	0	0	0	0	0	12	1	0	0	0	0	0	0	16	0	0	0	0	2
Simuliidae	Simu	1098	3851	2048	344	0	0	642	181	145	16	143	56	0	0	0	8	8	243	0	47	16	1	1	0
Siphlonurus	Siph	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	7	3	0	0	0
Sphaeridae	Spha	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stenelmis (larva)	Sten	33	21	31	59	2	31	25	62	274	0	3	0	1	5	0	174	253	97	0	79	16	0	17	4
Stratiomyidae	Stra	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	3	0	0	0
Tabanidae	Taba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Taeniopterygidae	Taen	0	0	0	12	0	0	0	1	24	0	2	0	0	0	0	0	0	0	0	0	0	175	0	0
Tanypodinae	Tanyp	24	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tanytarsini	Tanyt	464	301	20	260	55	122	57	61	184	0	4	167	33	28	3	312	97	120	62	71	4	20	38	116
Tipulidae	Tipu	137	174	83	25	0	6	27	13	22	16	17	12	1	0	4	3	2	6	0	8	0	15	2	0
Trichoptera (others)	Tric	1	28	12	0	0	0	8	0	0	0	1	0	0	0	0	0	0	0	6	1	1	1	0	0
Veliidae	Veli	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S4. Macroinvertebrate abundance in each sampling site in 2012.

	Abbrev	Sab1_12	Sab2_12	Sab3_12	Sab4_12	Sab5_12	Sab6_12	Sab7_12	Sab8_12	Sab9_12	Sab10B_12	Sab11_12	Sab12_12	Sab13B_12	Sab14B_12	Sab15_12	Xed3_12	Rel2_12	Moi2_12	Spe1_12	Sou2_12	Cal_12	Zac_12	Vil_12
Ancylus	Ancy	0	2	14	48	0	1	17	2	1	0	35	28	5	0	0	0	0	0	0	3	0	1	0
Athericidae	Athe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Atyaephyra	Atya	0	0	2	0	0	1	0	0	0	0	0	0	0	45	3	0	0	0	0	0	0	0	0
Baetidae (others)	Baet	0	2	0	5	0	0	61	8	0	59	122	1	0	0	0	68	310	50	0	19	107	10	1
Baetis	Baetis	1	12	175	88	0	2	248	87	27	0	127	64	7	0	6	11	220	52	0	1	616	37	0
Caenis	Caen	4	6	9	17	27	60	16	76	56	8	6	20	35	0	0	1	43	0	0	7	8	0	0
Capniidae	Capn	0	1	44	128	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Centroptilum	Cent	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	43	0	0	0	2	0	0
Ceratopogonidae	Cera	0	2	2	6	0	4	0	1	0	0	0	2	0	0	1	4	7	24	0	6	0	5	0
Chironomidae (others)	Chird	1	0	0	0	2	7	0	0	2	0	0	6	0	1	0	0	0	9	2	0	0	0	0
Chironomini	Chirn	3	1	10	2	21	23	0	16	5	0	0	27	20	2	3	14	60	51	353	7	4	2	5
Chloroperlidae	Chlo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0
Choroterpes	Chor	0	0	0	1	0	0	0	23	0	1	0	3	7	0	0	0	0	0	0	0	0	0	0
Cordulegasteridae	Cord	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0
Corixidae (others)	Cori	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	36	0	0	0	0	0	0
Corynoneura	Cory	0	0	0	1	0	0	0	0	0	0	36	0	4	0	0	16	0	11	0	14	35	2	0
Dicranota	Dicr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diptera n.i.	Dipt	0	8	18	17	3	3	36	7	18	6	88	73	12	3	4	26	11	12	13	4	19	2	3
Dixidae	Dixi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dryops (adult)	Dryad	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	6	3	0	0	1	0	0	0
Dryops (larva)	Dryla	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0
Dupophilus (adult)	Dupo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dytiscidae (adult)	Dytad	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	0	0	9	3	1	0	0
Dytiscidae (larva)	Dytla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	120	67	2	0	13	43	16	0
Ecdyonurus	Ecdy	0	0	5	21	0	0	5	14	1	0	15	2	1	0	1	0	0	0	0	57	3	4	0
Elmidae (others)	Elmi	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	1	1	14	0	0	0	0	0
Empididae	Empi	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	7	0	0	0	0	0
Ephemerella	Ephe	1	55	95	63	2	7	91	29	5	4	16	3	0	0	0	0	3	0	0	5	22	60	2
Ephoron	Epho	0	0	0	0	0	2	0	378	28	1	6	5	34	0	0	0	0	0	0	0	0	0	0
Erpobdellidae	Erpo	0	0	0	3	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	3	0
Gerridae	Gerr	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	7	1	0	5	0	0	0
Glossiphoniidae	Glossi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glossosomatidae	Glosso	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gomphidae	Gomp	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	13	0	4	0	0	0
Helodidae (larva)	Helod	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
Helophorus (adult)	Heload	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helophorus (larva)	Helola	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Heptageniidae (others)	Hept	0	0	0	0	0	2	0	3	0	0	1	0	0	0	0	0	0	6	0	0	0	0	0
Hexatoma	Hexa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hidracarina	Hidr	5	14	3	15	6	7	23	69	15	4	2	22	15	0	2	1	13	3	0	5	4	3	4
Hydraenidae (adult)	Hydrae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrobiidae	Hydrob	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	527	0	0	8
Hydrometridae	Hydrom	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	3	0	0
Hydrophilidae (adult)	Hydrad	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrophilidae (larva)	Hydrla	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	6	10	0	0	0	1	0	0
Hydropsychidae	Hydrop	0	2	7	39	0	0	152	38	2	6	469	16	4	0	0	0	0	7	0	0	2	3	0
Hydroptilidae	Hydrpt	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Isoperla	Isop	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	0
Leptoceridae	Lept	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptophlebiidae (others)	Leptph	0	0	0	3	0	0	0	4	0	0	3	2	4	0	2	0	20	135	0	51	31	126	3
Leuctridae	Leuc	0	0	0	10	0	0	1	2	0	0	0	0	0	0	0	0	0	5	0	3	0	0	0
Libellulidae	Libe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	4	0	0	0	7	0	0
Limnephilinae	Limn	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0	0	0
Limoniidae (others)	Limo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Micronecta	Micr	0	0	0	0	0	6	0	16	0	0	1	0	2	11	420	0	1	0	0	2	3	0	0
Naucoridae	Nauc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	11	0	0	0	0	0	0
Nemouridae	Nemo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nepa	Nepa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	2	1	0	0
Normandia (larva)	Norm	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Notonectidae	Noto	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	6	0	0	0	17	0	0
Oligochaeta	Olig	0	0	0	0	0	1	0	5	0	0	0	2	38	0	21	0	0	0	212	0	0	2	0
Oligoneuriidae	Oligne	0	0	2	0	0	0	1	0	0	0	51	1	0	0	0	0	0	0	0	0	0	0	0
Orthocladiinae	Orth	7	144	115	378	17	35	444	48	96	46	712	591	107	6	14	22	64	159	84	20	184	43	3
Oulimnius (adult)	Oulad	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	3	4	43	0
Oulimnius (larva)	Oulla	2	1	1	30	2	11	4	8	1	0	0	0	0	0	0	52	6	1	1	46	44	45	0
Paraleptophlebia	Para	0	0	0	0	0	0	0	22	14	0	2	2	3	0	1	0	0	1	0	0	0	1	0
Perlodidae (others)	Perl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	8	0
Philopotamidae	Phil	0	0	0	0	0	0	0	0	0	0	19	1	0	0	0	0	0	0	0	0	0	0	0
Physidae	Phys	0	3	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Planorbis	Plan	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	82	20	0	0	0	3	0	1
Polycentropodidae	Poly	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	2	0	0	0
Psychodidae	Psyc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0
Psychomyiidae	Psychm	0	0	1	2	0	0	0	7	2	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Rhyacophila	Rhya	0	0	0	2	0	0	3	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0
Scirtidae	Scir	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	104	0	0	0
Sericostomatidae	Seri	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0
Simuliidae	Simu	1	217	132	156	7	6	2536	1	14	12	772	202	7	1	0	1	8	5	2	0	470	4	1
Siphlonurus	Siph	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
Sphaeridae	Spha	0	0	0	0	0	0	0	0	3	0	1	0	0	0	0	0	5	0	0	0	0	0	0
Stenelmis (larva)	Sten	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0
Stratiomyidae	Stra	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tabanidae	Taba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Taeniopterygidae	Taen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tanypodinae	Tanyp	0	0	12	4	6	0	0	32	6	2	20	7	9	0	1	18	39	165	1	36	75	18	1
Tanytarsini	Tanyt	10	9	33	37	27	40	0	14	28	7	36	39	6	0	1	20	12	276	62	6	56	11	3
Tipulidae	Tipu	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
Trichoptera (others)	Tric	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	1	3	0	0	0	0	0
Veliidae	Veli	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S5. Macroinvertebrate abundance in each sampling site in 2013.

	Abbrev	Sab1_13	Sab2_13	Sab3_13	Sab4_13	Sab5_13	Sab6_13	Sab7_13	Sab8_13	Sab9_13	Sab10B_13	Sab11_13	Sab12_13	Sab13B_13	Sab14B_13	Sab15_13	Xed1_13	Xed3_13	Rel2_13	Moi2_13	Spe1_13	Sou1_13	Sou2_13	Cal_13	Zac_13	Poi1_13	Vil_13
Ancylus	Ancy	0	3	1	0	0	0	2	0	0	9	0	0	0	0	0	15	0	0	0	0	4	0	0	0	0	0
Athericidae	Athe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	7	0	0	0	0	0
Atyaephyra	Atya	1	0	0	0	0	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Baetidae (others)	Baet	68	81	209	5	6	1	237	20	5	34	15	6	0	0	4	14	62	288	170	0	44	70	3396	687	2	5
Baetis	Baetis	66	117	195	11	0	0	935	76	1	50	21	3	1	1	8	136	361	303	733	0	42	39	3436	933	0	6
Caenis	Caen	50	10	202	34	66	39	108	188	48	0	25	9	39	0	0	0	0	2	0	84	1	0	0	0	0	1
Capniidae	Capn	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Centroptilum	Cent	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ceratopogonidae	Cera	0	74	148	0	2	3	12	17	20	9	0	0	2	2	1	0	61	0	16	0	1	0	52	5	5	0
Chironomidae (others)	Chird	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chironomini	Chirn	136	57	8	200	144	173	20	93	48	26	0	22	67	11	6	54	2	77	15	26	0	0	0	0	1	2
Chloroperlidae	Chlo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	10	0	0	3	5	0
Choroterpes	Chor	0	0	0	0	0	0	0	2	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Cordulegasteridae	Cord	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	3	0
Corixidae (others)	Cori	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	47	0	0	0	0	0	0	0	0
Corynoneura	Cory	0	102	36	24	2	0	20	5	2	1	13	16	2	1	0	28	13	48	14	1	10	4	224	12	1	23
Dicranota	Dicr	1	8	1	0	0	0	0	0	0	0	0	0	0	0	0	1	8	3	0	0	2	19	8	10	3	1
Diptera n.i.	Dipt	116	113	12	24	12	12	112	26	31	92	74	37	17	22	3	0	4	28	7	14	0	2	256	2	0	23
Dixidae	Dixi	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	40	9	9	0	0	0	4	568	25	0	0
Dryops (adult)	Dryad	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dryops (larva)	Dryla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Dupophilus (adult)	Dupo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dytiscidae (adult)	Dytad	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	26	4	15	0	0	1	3	0	1	2	18
Dytiscidae (larva)	Dytla	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	36	0	140	0	0	9	8	36	75	4	3
Ecdyonurus	Ecdy	0	0	31	0	0	0	4	4	0	4	0	0	0	0	0	0	0	0	1	0	87	0	4	1	71	0
Elmidae (others)	Elmi	3	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	8	0	0	1	0	0	0	1	0
Empididae	Empi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	2	0	0	0	0	0	0
Ephemerella	Ephe	480	199	2194	35	7	16	462	79	63	85	63	30	4	0	0	0	0	3	0	0	15	2	412	734	51	2
Ephoron	Epho	0	0	0	3	0	1	18	334	61	154	4	27	181	0	0	0	0	0	1	0	0	0	0	0	0	0
Erpobdellidae	Erpo	0	1	0	0	0	2	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
Gerridae	Gerr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
Glossiphoniidae	Glossi	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	3	0	2	0	0	0	0	0	1	6	0
Glossosomatidae	Glosso	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	1	0
Gomphidae	Gomp	2	0	3	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	16	0	9	0	0	0	1	0
Helodidae (larva)	Helod	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helophorus (adult)	Heload	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	8	20	0	0	0	3	92	0	0	9
Helophorus (larva)	Helola	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heptageniidae (others)	Hept	0	1	0	0	0	0	0	3	0	0	0	1	0	0	0	0	0	0	0	0	18	1	0	0	1	0
Hexatoma	Hexa	0	0	1	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
Hidracarina	Hidr	31	1	0	37	25	62	37	162	303	89	30	49	312	3	5	0	8	8	4	0	14	2	40	84	0	10
Hydraenidae (adult)	Hydrae	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	9	8	8	16	0	3	2	49	0	1	2
Hydrobiidae	Hydrob	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	749	0	0	0	1	1
Hydrometridae	Hydrom	0	0	0	0	0	0	1	0	0	0	0	1	0	1	1	1	1	0	0	0	0	0	8	1	0	2
Hydrophilidae (adult)	Hydrad	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	20	0	0	1
Hydrophilidae (larva)	Hydrla	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	3	2	132	0	0	0	0	20	0	0	0
Hydropsychidae	Hydrop	52	41	240	0	1	0	0	0	0	10	4	1	0	0	0	2	0	0	0	0	0	0	4	1	0	1
Hydroptilidae	Hydrpt	0	0	0	0	1	0	0	1	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	8
Isoperla	Isop	2	1	5	0	0	0	0	0	0	0	0	0	0	0	0	2	58	18	0	0	4	42	116	28	13	0
Leptoceridae	Lept	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptophlebiidae (others)	Leptph	1	0	1	2	0	2	15	199	62	24	2	33	127	0	0	0	0	1	159	0	173	0	0	17	53	11
Leuctridae	Leuc	72	84	176	0	0	1	0	1	1	2	0	0	0	0	1	0	0	0	71	0	114	0	0	1	18	0
Libellulidae	Libe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0
Limnephilinae	Limn	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	47	0	4	0	0	0	0	0
Limoniidae (others)	Limo	0	72	0	0	0	0	0	0	0	0	0	0	0	4	1	0	0	0	0	0	9	1	0	0	1	2
Micronecta	Micr	0	0	0	0	40	0	34	4	0	0	0	0	0	3	64	0	0	0	0	0	0	0	0	0	0	0
Naucoridae	Nauc	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nemouridae	Nemo	0	0	3	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nepa	Nepa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	4	3	0	0
Normandia (larva)	Norm	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
Notonectidae	Noto	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	26	0	0	0	0	0	0	0	0
Oligochaeta	Olig	5	1	13	52	30	8	26	83	21	15	20	92	38	61	303	1	15	8	25	12	0	0	8	0	0	19
Oligoneuriidae	Oligne	1	4	9	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Orthocladiinae	Orth	2776	1081	2640	652	86	123	808	128	306	611	292	386	112	44	14	250	161	159	127	76	37	115	1796	71	27	285
Oulimnius (adult)	Oulad	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	38	31	1	0	18	5	160	132	0	0
Oulimnius (larva)	Oulla	0	0	85	10	5	23	46	36	89	19	14	9	26	0	0	98	76	110	0	0	0	9	1092	593	1	0
Paraleptophlebia	Para	0	0	0	0	0	0	0	46	12	8	0	4	10	0	0	0	0	4	0	0	0	0	0	8	0	5
Perlodidae (others)	Perl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0
Philopotamidae	Phil	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Physidae	Phys	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	40	6	1	0	2	4	0	0	0
Planorbis	Plan	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	54	1	41	0	0	0	0	20	1	0	0
Polycentropodidae	Poly	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	5	0	0	0	1	0
Psychodidae	Psyc	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	8	0	0	0
Psychomyiidae	Psychm	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	8	0	0	0	0	0	0	0	0
Rhyacophila	Rhya	4	31	35	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	11	0	0	0	12	0	0	0
Scirtidae	Scir	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	7	10	0	19	0	6	1	12	0	0	0
Sericostomatidae	Seri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0
Simuliidae	Simu	13488	1299	11484	88	0	7	8	2	6	322	957	16	0	1	0	194	0	1	33	7	1	152	2832	161	1	8
Siphlonurus	Siph	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sphaeridae	Spha	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	18	0	0	0	0	0	0
Stenelmis (larva)	Sten	0	2	2	0	0	0	0	6	0	0	0	0	0	0	0	16	0	0	0	0	15	0	0	0	0	0
Stratiomyidae	Stra	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tabanidae	Taba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Taeniopterygidae	Taen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tanypodinae	Tanyp	36	0	56	40	21	4	12	25	13	18	22	6	9	1	0	136	5	178	140	102	61	27	176	376	14	8
Tanytarsini	Tanyt	136	77	40	48	20	30	88	27	4	20	38	82	45	14	2	110	70	313	69	368	4	2	48	4	2	41
Tipulidae	Tipu	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	0	0	0	0	15	0	0	0	0	0
Trichoptera (others)	Tric	0	8	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	0	1	0	2	0	0	0	0	1
Veliidae	Veli	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	2

CAPÍTULO III

Long-term effects of a metal-bearing effluent in freshwater benthic macroinvertebrates

Abstract

S. Domingos mine (southeast Portugal) is a typical “abandoned mine” with all sorts of problems as consequence of the cessation of the mining activity and lack of infrastructure maintenance. The mine is closed at present, but the heavy metal enriched tailings remain at the surface in oxidizing conditions, resulting in the rapid formation of a highly acidic and metal rich leachate, acid mine drainage, which can seriously impact surrounding freshwater systems. Biotic community assessments are useful tools to monitor such changes, and the European Water Framework Directive (WFD) has brought this into the legislative framework. In this scenario, the main aim of the present study was to assess the potential effects of chemical stressor (acid mine drainage) on benthic macroinvertebrate communities, through a water quality approach, focusing on ecological status. Five stations in the aquatic system surrounding S. Domingos mine were selected for this study, during the spring, in 2006 and 2014: three reference sites (TG, TP and CP) and two impacted sites (ED and CD). The results contrast with our *a priori* expectations with all sampling sites presented low levels of conductivity and dissolved metals and neutral pH, which suggests that the degree of contamination is, at least currently, low. Nevertheless, the WFD approach concluded that for both years all sample sites had a less than good ecological status. The use of generic metrics, such as richness and EPT *taxa*, which were below the reference value for all stations, could produce confounding results, and may either over- or understate environmental quality. The solution to this problem could be the use of fine-resolution community analysis tools. This may improve the discriminatory power of bioassessments and aid in identifying the causal nature of the observed impacts.

Key Words: Water Framework Directive, São Domingos mine, acid mine drainage, water pollution/ water chemistry, chemical stressor, benthic macroinvertebrates.

Introduction

High levels of trace elements in freshwater bodies may occur as a result of natural weathering of minerals in the sediments and bed rocks or as a result of anthropogenic activities such as mining, industrial, municipal and agricultural discharges (Siwela, Nyathi, & Naik, 2010). Because of their environmental hazardous potential towards recipient aquatic ecosystems and especially metal-sensitive groups such as crustacean and mayflies (Malmqvist & Hoffsten, 1999), mining activities have been receiving significant attention. Mineral resources have been exploited for thousands of years and the majority of the mine wastes have a negative visual impact on the landscape due to tailings volume, huge heap dimensions and unsuitability for vegetation growth (Santos, Abreu, Nabais, & Magalhães, 2012). Deactivated mines can also be responsible for several impacts in the surrounding environmental compartments due to the chemical nature of their effluents, which are often left unattended for long periods of time as a result of inexistent or inefficiently applied recovery plans for the area (Lopes, Gonçalves, Soares, & Ribeiro, 1999). These effluents consist of acid mine drainage (AMD), i.e. eroded material from mine tailings and waste rocks (Gomes, Antunes, Silva, Neiva, & Pacheco, 2010). AMD is formed when pyritic rocks and ores are exposed to atmospheric oxygen during the extraction of metal cores (e.g. Fe, Cu, Zn, Pb, As, U), sulphur or coal mining (Gray, 1998). Water and oxygen promote bacterial-mediated oxidation of exposed rocks and minerals (Hudson-Edwards, Jamieson, & Lottermoser, 2011), this resulting in the rapid formation of a highly acidic, metal- and sulfate-rich leachate that can seriously impact both surface and ground waters in the surroundings (El Khalil, El Hamiani, Bitton, Ouazzani, & Boularbah, 2008).

In fact, AMD is the main pollution process of natural watercourses in the Iberian Pyrite Belt (Rodríguez-Jordá, Garrido, & García-González, 2012), which extends for about 250 km crossing Portugal and Spain and it is one of the most important volcanogenic massive sulphide areas in the world (Varennes, Qu, Cordovil, & Goncalves, 2011). Human settling and economic development in this area was largely dependent on mining activities, which were especially intense from the middle of the 19th century onwards (Pérez-López, Delgado, Nieto, & Márquez-Gárcia, 2010). The area was exploited for Au, Cu and Ag during pre-

Roman and Roman times, and modern extraction of Cu and Zn still takes place at some sites. On the other hand, more than 100 mines were closed during the 20th century due to ore exhaustion. The lack of appropriate legislation and regulatory guidelines addressing environmental impacts of mining activities has been allowing that the mine tailings are left without any treatment or containment: large areas, both in Portugal and Spain are thus presently contaminated with a wide range of trace elements including Cu, Pb, As and Zn, representing the world's largest area of sulphide generated wastes (Varennes et al., 2011).

The effect of AMD on rivers is dependent primarily on the dilution effects within each particular system but also on the ecosystem's buffering capacity. However, dedicated studies so far indicate consistently that AMD is able to promote negative impacts in lotic systems, including pH decrease, raised metal concentrations (e.g. Fe, Zn, Cu, Al, Pb, As, Cd, Mn, Se), ochre formation, which is a stable orange precipitate comprising iron oxyhydroxides, and increased sulphate concentration (Gray, 1996; DeNicola & Stapleton, 2002; Akcil & Koldas, 2006). The typical AMD-driven acidity, metal toxicity, metal precipitation and salinization of rivers (Gray,1997) generally promote threefold effects in the ecosystems, namely (i) impaired biological communities, in particular decreased biota richness and diversity; (ii) alterations in nutrient cycles and abiotic changes, such as the nullification of the bicarbonate buffering capacity of the acidified system and (iii) biological communities becoming restricted to tolerant organisms able to survive under these extreme conditions (Rosenberg & Resh, 1993; Gray, 1997; Oberholster et al., 2013).

In order to assess and manage the potential impact that could result from the dispersion of chemical contaminants into aquatic ecosystems, a certain number of measures and regulations have been set up by EU member states (Guérit, Bocquene, James, Thybaud, & Minier, 2008). In this context, the Water Framework Directive (WFD) boosted the development and implementation of reference conditions, monitoring schedules and plans, assessments of pressures and impacts, towards the achievement of a golden target of reaching ‘‘good ecological and chemical status’’ in all European waterbodies first before 2015, then postponed to 2027. In this way, the standard assessment of the status of a river *sensu* WFD has been set as a comprehensive protocol through the integration of different lines of assessed evidences, e.g. biological, physic-chemical and hydro-morphological (EU, 2000). For example, it is standard practice to assess biological evidences of river ecological status by centring the analysis on benthic macroinvertebrate communities. They have the advantage of being relatively sedentary and thus representatives of the conditions within each sampled site. They are diverse, reflecting a range of potential tolerance to changing environmental conditions, including chemical contamination. Their life cycles vary from intra-annual to inter-annual so that they can integrate antecedent conditions from short-term episodes to longer term changes (Iwasaki & Ormerod, 2012).

Despite this bioindicator value, auto-ecological information for most macroinvertebrate *taxa* comprises information on their tolerance to organic, difuse pollution but not, regarding other types of stressors (Dahl, Johnson, & Sandin, 2004; Feld, 2004; Lorenz, Hering, Field, & Rolauffs, 2004; Sandin & Hering 2004; Vlek, Verdonschot, & Njiboer, 2004). Indeed, WFD metrics rely on biotic indices that were mostly designed to signal organic pollution, such as the EPT (number of taxa belonging to the orders Ephemeroptera, Plecoptera and Trichoptera) or ASPT (average score per taxon, derived from the biological monitoring working party (BMWP); Chapman & Jackson, 1996). On the other hand, benthic macroinvertebrates are generally seen as the most sensitive indicators of metal contamination (Rosenberg & Resh, 1993), especially the metal-sensitive groups such as crustaceans and mayflies (Malmqvist & Hoffsten, 1999) (see above).

This study is focused on the long-term variation of the benthic macroinvertebrate community across a gradient of AMD contamination yield from a deactivated cupric mine in Portugal that belongs to the Iberian Pyrite Belt, the S. Domingos mine. The large scale exploitation of this deposit occurred between 1857 and 1966, when pyrite and sulphides of several trace elements became exposed to the air and were responsible for the pollution observed in soils, superficial water and sediments, mainly through water erosion and eolian dispersion (Alvarenga, Palma, Varennes, & Cunha-Queda, 2012).The exploitation ceased in 1968, but the mine is still active from the contamination point of view due to the continuing drainage of AMD into surrounding freshwater systems (Rodríguez-Jordá et al., 2012). In fact, although the environmental rehabilitation of the area was rendered to a public company (Empresa de Desenvolvimento Mineiro, S.A) and initiated in 2003. The process is currently suspended at an early stage mostly due to budget limitations and divergences between stakeholders on the potential uses for the complex in a post-mining stage (Sardinha, Craveiro, & Milheira., 2013). This certainly contributes to the assignment to the highest level of environmental danger by a study surveying 85 abandoned mines in Portugal (Oliveira et al., 2002).

The present study intended to evaluate the ecological status of S. Domingos mine area by using the WFD approach based on the macroinvertebrate community. To accomplish this general aim, two approaches were used: (i) sensitivity of macroinvertebrates to the AMD (chemical stressor) and (ii) assessment of the ecological status of the study area in two different periods (2006 and 2014).

Material and Methods

Study area and sampling strategy

São Domingos mine was one of the most emblematic Portuguese mining complexes located in Mértola (Beja District, southeast of Portugal) and characterized by a Mediterranean mesothermic humid climate, with hot dry summers and short winters (Alvarenga et al., 2012). It contains ores of pyrite, chalcopyrite, sphalerite and galena (MINEO, 2003). The oxidation of this pyrite environment produces an effluent (AMD) with high concentrations of heavy metals (Al, Fe, Zn, Cu, Mn, Co, Ni, Cd, Cr, Pb, and As) and with very low pH (Lopes et al., 1999), which flows to the South and joins the Chança river, a main tributary of the Guadiana river (Pérez-López, Delgado, Nieto, & Márquez-Gárcia, 2010). In São Domingos the drainage of the mining area occurs along several kilometres until the Mosteirão stream, a Chança River tributary. A system of channels, dams and ponds was built for retention, sedimentation and evaporation of the AMD in order to prevent spreading of contamination (Pereira, Soares, Gonçalves, & Ribeiro, 1999). Nowadays, the abandoned mine tailings still continue to be a large and unsolved environmental problem due to the continuous leaching of mine residues (Álvarez-Valero et al., 2008; Pérez-López et al., 2008).

Five stations within the aquatic system surrounding S. Domingos mine were selected for this study, the sampling occurring the spring, in 2006 and almost ten years late, in 2014; three reference sites (Tapada Grande - TG, Tapada Pequena - TP and Corte do Pinto - CP) and two impacted sites (English Dam - ED and Chança Dam - CD) were covered (Figure 1). TG and TP were located in independent semiartificial lagoons, with no history of contamination by metals and far away from the effluent discharge. They were built by the mining company to provide water to mining activities. Nowadays, TG and TP are highly demanded for recreational activities. CP stands in a village with the same name located in the north of the mine, thus upstream AMD inputs. The Chança River has its headwaters in the Aracena Mountains (Spain), and flows for 96 km until its confluence with Guadiana River in Pomarão (station CD). Upstream the confluence, a large dam was built in 1985 creating a new reservoir – the Chança Reservoir (station ED). This reservoir is used to supply water for municipal and agriculture uses (Quental et al., 2002).

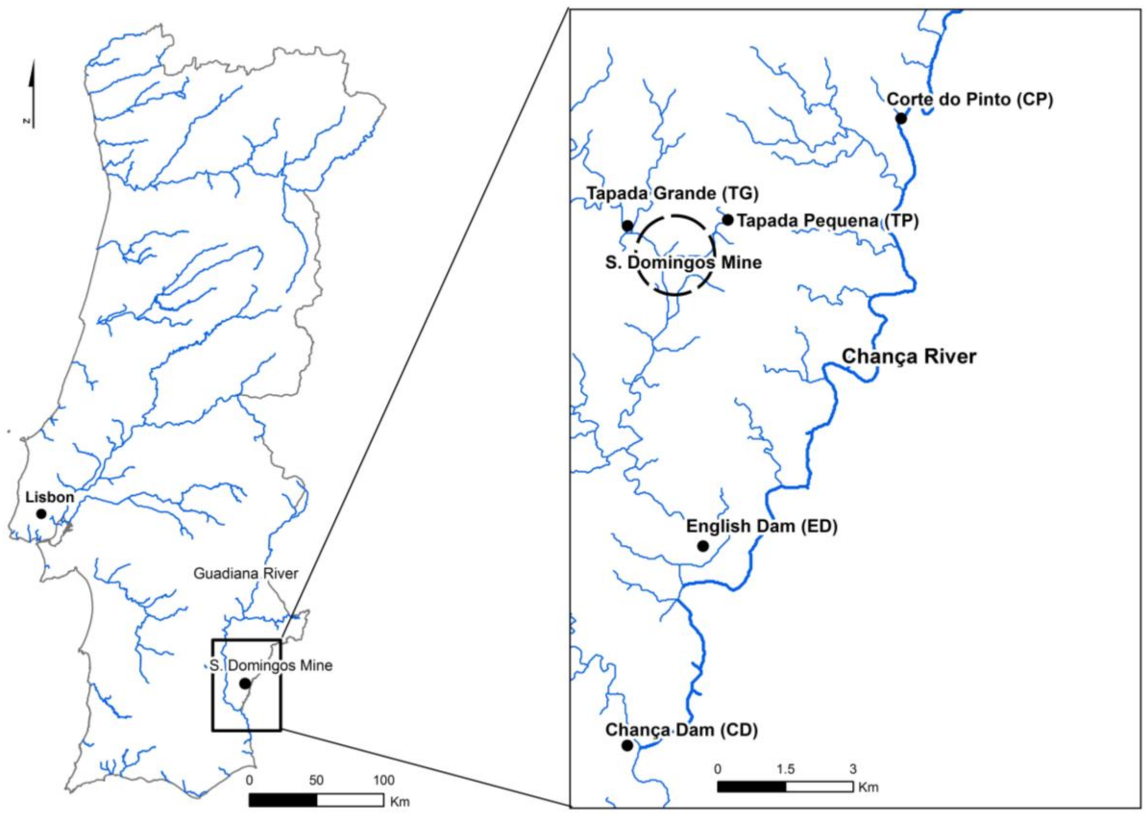


Fig. 1. Schematic map of the study area with the sampling sites.

Physicochemical analysis

At each sampling site, temperature ('Temp'), pH, dissolved oxygen ('Oxi') and conductivity ('Cond') were measured *in situ* with a multiparameter field probe (WTW-Multi3430). Water was collected for metal analysis following immediate acidification with nitric acid (65% pro analysis) to pH <2 and short storage at 4°C. Total concentrations of Al, Ba, Ca, Cu, Fe, Mg, Mn, Na, Ni, Pb, Si, Sr and Zn were quantified in these water samples. All analyses were performed externally by an accredited laboratory (DIN EN ISO/IEC 17025 notification under the DAkkS German Accreditation System for Testing; ISO, 2005). The quantification of Al, Ba, Cu, Mn, Ni and Pb in water samples, was performed by atomic absorption spectrometric (APHA, 1999); Ca, Mg, Na and Zn by flame atomic absorption apectrometry (APHA, 1999); Fe by molecular absorption spectrometric (APHA, 1999); and Si and Sr by TP (Technical Procedures) 15.

Macroinvertebrates sampling and processing

Macroinvertebrates were collected at each sampling site by kick-sampling small transects, using a standard hand net (0.5 mm mesh size; square frame 0.30 x 0.30 m). Macroinvertebrate sampling was made according to the proportional presence of microhabitats (Hering et al., 2003; INAG, 2008). The collected samples were placed in air-tight plastic containers and preserved with 80-90% ethanol (Barbour, Gerritsen, Snyder, & Stribling, 1999) for further analysis. In the laboratory, preserved benthic macroinvertebrate samples were washed through a 500-µm-mesh size sieve. Sorted organisms were counted and identified to the lowest practical taxonomical level, generally the family (or genus, when possible) using general and taxon-specific identification keys (Macan, 1959; Richoux, 1982; Tachet, Bournaud, & Richoux, 1980; Sundermann, Lohse, Beck, & Haase, 2007; Serra, Coimbra, & Graça, 2009).

Data analysis under WFD approach

<u>Chemical and physicochemical elements</u>

In the the physicochemical analysis, were used the values for class Good for Portugal, referred in INAG and Decree-Law 236/98 of August 1 (DL; aquaculture, bathing water and irrigation water). The trace elements concentrations were compared with the reference values established not only in INAG and DL, as well as the Water Quality Criteria (WQC; USEPA, 2009).

<u>Biological quality elements</u>

The sampling sites (except CP) are not in a lotic system and according to the WFD, the biological elements benthic macroinvertebrates have been established only for rivers. In this context, the WFD does not include an integrated study river-reservoir; the data were analyzed by comparing the different locations and years, taking into account the benchmarks set by the directive.

Community metrics were calculated for the macroinvertebrate community samples, using family as the taxonomical resolution level: total number of families (richness, S), Shannon's diversity index (H'), Pielou's equitability index (J'). Three biotic indices were also calculated: i) EPT is the number of *taxa* belonging to orders Ephemeroptera, Plecoptera and Trichoptera; ii) IASPT (Iberian Average Score per *Taxon*) is the average score per taxon, derived from the biotic index IBMWP (Iberian Biological Monitoring Working Party), which is specifically adapted to the Iberian Peninsula (Alba-Tercedor & Sánchez-Ortega, 1988); and iii) log(sel. EPTCD + 1) is the logarithm (plus one) of the sum of abundances of organisms belonging to families Chloroperlidae, Nemouridae, Leuctridae, Leptophlebiidae, Ephemerellidae, Philopotamidae, Limnephilidae, Psychomyiidae, Sericostomatidae, Elmidae, Dryopidae, Athericidae. Some of these metrics were informative *per se*; however, they were mostly calculated for later integration in the South Invertebrate Portuguese Index ($IPtI_S$) multimetric index, as follows:

$$\mathbf{IPtI_S = (S \times 0.4) + (EPT \times 0.2) + ((IASPT - 2) \times 0.2) + (Log\ (Sel.\ EPTCD+1) \times 0.2))}$$

$IPtI_S$ allows the derivation of Ecological Quality Ratios (EQRs) for the classification of ecological quality for each sample (following the WFD and INAG technical papers; INAG, 2009). Reference values for the community metrics and biotic indices were obtained from official guidance documents (INAG, 2009), considering Chança river as a type S1≥100 river – rivers with a catchment area ≥100 km^2.

Results and Discussion

The study area was expected to suffer the effects of AMD, either at specific locations or along its extension. AMD can persist for centuries after the deactivation of a mine, contaminating soils and water in the surrounding as well as sites further downstream (e.g., Byrne, Reid, & Wood, 2013). In fact, S. Domingos is one of the deactivated mines in Portugal bearing higher environmental hazard potential, especially due to AMD (Matos, Soares, & Cardoso, 2006). According to these authors, with the cessation of mining activity, the open pit was progressively flooded by acid waters (pH below 3); high concentration of metals can be found in the tailings deposited near the open pit throughout the various mining seasons, which have been dragged - or leachate - per share of rainwater, contaminating a nearby river (Mosteirão stream, tributary of the Chança river).

Chemical and physico-chemical elements

The physicochemical properties of water from São Domingos sampling sites are presented in Table 1. There were no major fluctuations within sampling sites through the time. However, higher temperatures, conductivity and pH were recorded in 2014 for all sites; whereas dissolved oxygen was notably higher in 2006, with the exception for site TG. Conductivity values were always low in both years and sites (below 1000 µScm^{-1}, see Table 2), and there is no apparent association between higher conductivity and putatively impacted sites (ED and CD). The same is retrievable from pH records since waters from ED and CD were neither acidic in general nor more acidic than those from reference sites (Table 1). Dissolved oxygen was almost always higher than 8 mgL^{-1} regardless the site or the sampling year. All values of physico-chemical parameters are according to reference values for freshwater systems (Table 2).

Table 1. Physicochemical water parameters for the sampling sites.

	TG		**TP**		**CP**		**ED**		**CD**	
	2006	**2014**	**2006**	**2014**	**2006**	**2014**	**2006**	**2014**	**2006**	**2014**
Temp (ºC)	19.2	27.1	19.7	29	18.9	28.7	18.5	24.7	18.7	25
Cond (µScm^{-1})	268	314	453	464	325	327	262	557	263	480
pH	7.16	8.39	7.72	8.05	7.63	8.35	7.12	7.97	7.24	8.21
Oxi (mgL^{-1})	6.9	7.99	12	8.41	9.3	7,89	9,4	8.99	9.2	8.51

Table 2. Reference value for chemical and physicochemical water parameters by INAG (2009), Decreet Law (DL) nº 236/98 and USEPA (2009); MRV – maximum recommended value; MAV – maximum admissible value.

	INAG	**DL (MRV/MAV)**			**WQC**
		Aquaculture	Bathing waters	Irrigation	
Temp (ºC)	-	-/-	-/-	-/-	-
Cond (µScm^{-1})	-	-/-	-/-	1000/-	-
pH	6-9	-/-	-/6-9	6.5-8.4/4.5-9	-
Oxi (mgL^{-1})	≥5	-/-	-/-	-/-	-
Al (µgL^{-1})	-	-/-	-/-	5000/20000	-

Ba (μgL^{-1})	1000	-/-	-/-	1000/-	-
Cu (μgL^{-1})	100	112/-	-/-	200/5000	130
Fe (μgL^{-1})	-	-/-	-/-	5000/-	-
Mn (μgL^{-1})	-	-/-	-/-	200/10000	-
Ni (μgL^{-1})	-	-/-	-/-	500/2000	470
Pb (μgL^{-1})	-	-/-	-/-	5000/20000	65
Si (μgL^{-1})	-	-/-	-/-	-/-	-
Sr (μgL^{-1})	-	-/-	-/-	-/-	-
Zn (μgL^{-1})	-	2000/-	-/-	2000/10000	120
Ca (μgL^{-1})	-	-/-	-/-	-/-	-
Mg (μgL^{-1})	-	-/-	-/-	-/-	-
Na (μgL^{-1})	-	-/-	-/-	-/-	-

Trace elements concentrations (Al, Ba, Cu, Fe, Mn, Ni, Pb, Si, Sr, Zn, Ca, Mg and Na) found in the sampling site are shown in Table 3. In general, most metal concentrations were low in water compartiment, when compared with reference values (Table 2). However, Ca, Mg and Na (components of total hardness) were found at higher concentrations (above 2000 μgL^{-1}). TP (only 2014), CP, ED (only 2006) and CD were also the stations with Si at concentrations above 2000 μgL^{-1}. Contrarily to what could be expected CP, a reference site located far away from S. Domingos mine, and CD, an impacted site situated downstream the Chança river, were not the sites with the lowest and the highest metal levels, respectively.

The results also showed no consistent pattern through time within each trace element. Concentrations of Pb, Mg, Ni, and Zn decreased with time, in all sample sites, as well as Ba, Ca, Si and Sr in TP, CP and ED. Al, Cu and Fe were generally found at higher concentrations more recently than in 2006. In 2006, TP (an independent semiartificial lagoon) revealed highest concentrations of Al, Ba, Sr and Fe when compared to the other semiartificial lagoon (TG). The opposite was observed in 2014. For the other three sampling stations, in 2006, the highest concentrations of Al, Ba, Cu, Fe, Mn and Ni were found in the putative reference site CP, with CD revealing the highest burden in other metals. In 2014, Al, Mn and Zn levels were the highest in ED (located in Chança reservoir) and Ba, Sr, Fe and Si in CD.

The toxicity of metals can be altered in natural waters by site-specific biogeochemical factors. A well-known modifying factor of toxicity for many metals (e.g., Cu, Cd, and Zn) is the total hardness of the water, which often correlates well with alkalinity (Welsh, Lipton, Chapman, & Podrabsky). Ca and Mg ions may inhibit trace metal adsorption to particles through competition for particulate binding sites. Although these ions generally form weaker complexes than trace metals, they are present in natural waters at concentrations much greater than that of trace metals, increasing the likelihood of the competition for binding sites (Lu & Allen, 2001). These ions also can decrease the bioavailability of Cd, Cu and Zn. This hardness effect is thought to result from the competition of Cd and Zn with Ca and Mg ions for binding sites (Adeni, Yusuf, & Okedeyi, 2008).

Table 3. Water metal concentrations (μgL^{-1}) measured at the sampling sites (TG, TP, CP, CP, ED and CD) in S. Domingos mine area, in both campaigns (2006 and 2014), BQL – Below Quantification Limit.

	TG		**TP**		**CP**		**ED**		**CD**	
	2006	2014	2006	2014	2006	2014	2006	2014	2006	2014
Al	36.9	510	141	71	365	160	66.1	260	46.4	170
Ba	11.6	14	42.5	BQL	16.4	BQL	14.0	BQL	12.7	15
Cu	1.40	24	1.39	13	12.2	29	10.7	4.1	9,86	3.3
Fe	101	870	369	150	497	260	112	190	70,6	270
Mn	54.2	66.2	131	7.1	91.9	22,6	34.0	35.6	29.2	19.6
Ni	0.77	BQL	1.09	BQL	1.31	BQL	1.29	BQL	1.28	BQL
Pb	0.40	BQL	3.42	BQL	1.07	BQL	0.54	BQL	0,98	BQL
Si	529	BQL	64.2	2100	2144	2200	2911	BQL	2388	3000
Sr	43	93	113	48	71.7	46	51.7	49	57.8	66
Zn	1.00	BQL	5.55	BQL	31.8	BQL	23.6	BQL	31.1	BQL
Ca	8450	39900	29800	19600	24300	19800	16374	8300	17057	20300
Mg	8200	3020	13700	3000	12468	2690	9445	2970	10042	3020
Na	35177	44800	46700	18100	25688	18400	20995	36400	20657	22300
Total*	779.3	1577	872.1	2389	3232	2718	3225	538.7	2567	3544
Total	52605	89297	91067	43089	65657	43608	50015	48209	50291	49164

* Excludes Na, Ca and Mg concentrations (components of water hardness)

The type of metals occurring in the acid drainage is dependent on the nature of sulphide minerals (Bryan, Hallberg, & Johnson, 2006; Costa & Duarte, 2005). Iron, copper and zinc and, to a lesser extent, cadmium, lead and arsenic, were the metals found in highest quantities dissolved in the water column, which is a common feature of waters surrounding mines draining massive sulphide deposits (España et al., 2005). Aluminium and manganese were also elements found in high quantities in the dissolved fraction. The geochemical study by Bryan et al. (2006) also identified copper, zinc and iron as the most readily mobilized metals in São Domingos mine wastes. However, the characteristics of ED, located downstream the confluence with the Mosteirão stream (Chança reservoir), and CD, standing downstream the Chança River were not consistent with the expected for impacted mining areas. These sites recorded low levels of conductivity and metals and neutral pH. Thus non-alarming picture can be related to the dynamics of the Chança reservoir system, highly dependent on climatic conditions, e.g. pluviometry (Álvarez-Valero et al., 2008; De Bisthoven, Gerhardt, & Soares, 2004). In fact, raining events influence the water level in the channels and reservoirs along the excavated valley where the acid drainage flows down towards the Mosteirão stream. Rainfall data recorded in Guadiana basin area (meteorological station of Serpa) between october 2005 and september 2006 and, october 2013 and september 2014 were 81.2 mm and 93.5 mm, respectively. However, during the study period, in 2006 rained (55 mm) but there was a lack of precipitation in 2015 (APA, 2016). Moreover, before entering into the Chança River reservoir the mine effluent is slightly diluted with water coming from the Mosteirão stream, resulting in the decrease of dissolved metal concentrations (Langmuir, 1997). Contamination of Mosteirão and Chança surface waters by metals (especially As, Cd, Cu, Mn, Ni, Pb, Co, and Zn) is documented in previous studies conducted in the area (De Bisthoven et al., 2004; Lopes et al., 1999; MAOT, 2001; Pereira, Soares, Gonçalves, & Ribeiro, 2000). Besides metals no other significant contamination sources are known (e.g., pesticides, industrial discharges or urban runoffs) in the Chança reservoir, as agriculture is scarce and rudimentary, industrial activity is inexistent, and the demographic density of the area is amongst the lowest in Europe (MAOT, 2001; Pereira et al., 1999; Pereira, Ribeiro, & Gonçalves, 2004).

Biological quality elements

In all samples, the number of families (richness) and number of sensitive *taxa* (Ephemeroptera, Plecoptera and Trichoptera – EPT) were below the reference value for the corresponding river typology (21 and 9, respectively), suggesting impacts from putative contamination sources. Surprisingly, the three referenced stations were also included in this context (Figures 2 and 3). In 2006, the benthic macroinvertebrate assemblage recorded maximum values of richness at CP, the putative reference site upstream the mine, and minimums at CD and ED, putatively impacted by AMD. Nonetheless, the lowest richness values was achieved for CP in 2014. However, this metric was quite variable through time. In TP, ED and CD sites, the richness increased from 2006 to 2014 and the remaining sites showed a decrease (Figure 2). Species richness is a fundamental measurement of community and regional diversity, and it underlies many ecological models and conservation strategies. In spite of its importance, ecologists have not always appreciated the effects of sampling effort and abundance on richness measures and comparisons (Gotelli & Colwell, 2001). It has been shown that richness values increases with the number of samples taken from different *habitats* (Bady et al., 2005). Bady et al. (2005), showed that five to ten samples were sufficient for estimating the species richness. In our study, this metric was not affected by sampling effort, with 6 samples (defined by INAG (2008)) taken for each site. On the other hand, the low

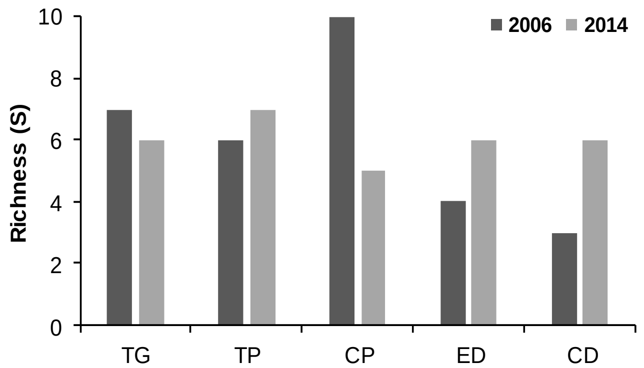


Fig. 2. Specific richness for each sampling station, in each sampling period.

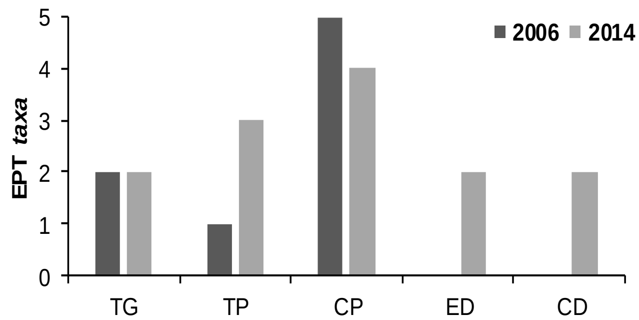


Fig. 3. EPT *taxa* for each sampling station, in each sampling period.

abundance decreases richness species (Gotelli & Colwell, 2001), because fewer individuals were collected in the sites. In 2006, EPT *taxa* were only present in the three first stations, namely TG, TP and CP (reference sites), while, in 2014, they were found in all sample sites. The highest EPT *taxa* value was recorded for CP for both sampling periods, although there was a decrease within site through time (Figure 3). There are no specific indicator species for AMD in affected rivers, although oligochaetas and dipterans, and chironomids in particular, are generally the dominant macroinvertebrate groups found downstream of mine drainage inputs; on the contrary, ephemeropterans are particularly sensitive to it and are amongst the last groups to recolonize rivers after contamination (Gray, 1997). Plecoptera are also considered to be generally sensitive to metals, whereas Trichoptera are relatively tolerant (Malmqvist & Hoffsten, 1999; Hickey & Golding, 2002). Contrarily, the results revealed that in all sampling sites (impacted and non-impacted locals) dipterans (especially chironomids) and ephemeropterans (sensitive *taxa*) were the dominant groups, while plecopterans (sensitive *taxa*) were never found (Table S1).

All sampling sites had a less than good ecological status (water quality based on $IPtI_S$-derived EQR values). In general, there was a slightly increase in ecological quality through time, with ecological status improving from bad to poor in TP, ED and CD (Figure 4). Although small, this increase of quality from bad to poor was due the increase in number of families (richness) and EPT *taxa* (Figures 2 and 3, respectively), but also other community metrics (e.g. diversity). In 2006, the highest EQR was achieved by CP, but the moderate ecological quality was depressed through time, and CP recorded poor ecological quality in 2014. In fact, in 2014, the ecological status of the whole sampled system (putative references and impacted sites) was invariably poor.

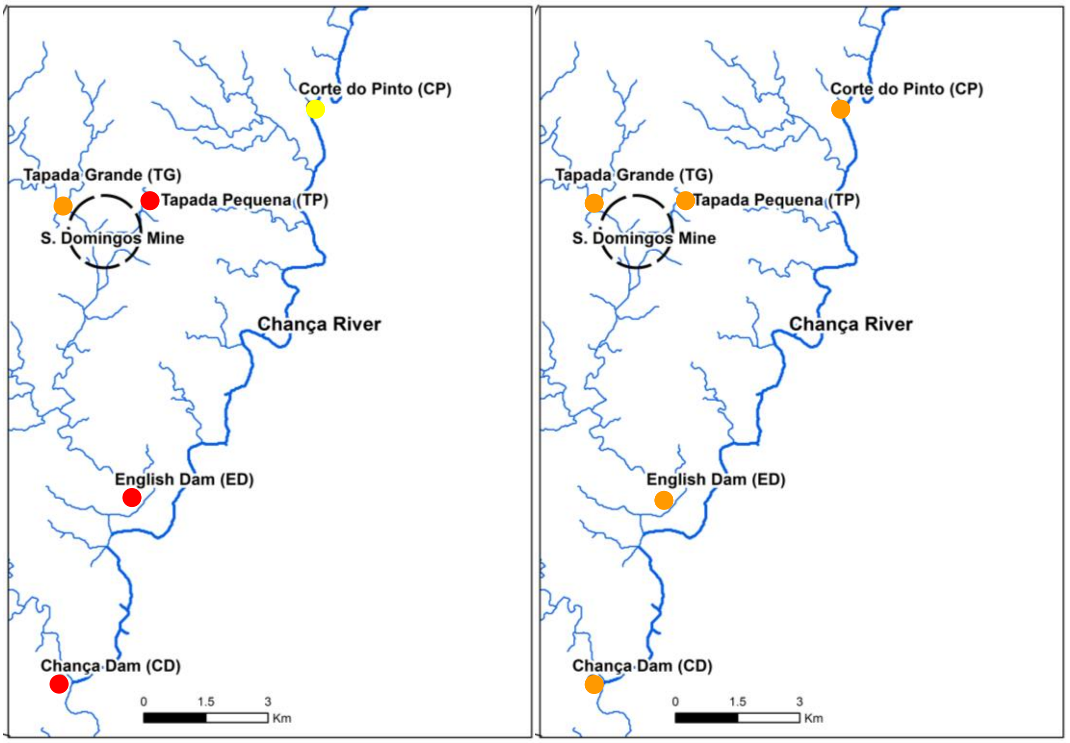


Fig. 4. Ecological status of the sampling sites in 2006 and 2014, respectively, according to EQRs values (● - bad; ●- poor; ● - moderate).

Several studies have shown that some of the metrics used in the WFD approach (richness, EPT taxa) are sensitive to metal contamination (Hickey & Clements, 1998; Mebane, 2001; Beasley & Kneale, 2003), particularly in mining areas (Hoiland, Rabe, & Biggam, 1994; Maret, Cain, MacCoy, & Short, 2003; Iwasaki, Kagaya, Miyamoto, & Matsuda, 2009). However, natural factors, such as elevation (Clements & Kiffney, 1995) or fine-sediment accumulation (Mebane, 2001), also change the response of such metrics. Additionally, Carlisle & Clements (1999) showed that the sensitivity of certain metrics (EPT *taxa*) in experimental streams did not correspond to field observations, most likely because of differential sensitivity to metals between mayflies and other EPT *taxa* (Clements & Kiffney,1995). Interestingly, the variability and accuracy of some indices have been shown to be highly dependent on the variability in species abundances (Diaz, Solan, & Valente, 2004). Therefore, the use of generic metrics will always produce confounding results, and may either over- or understate environmental quality. The solution to this problem could be the use of fine-resolution community analysis tools. This may improve the discriminatory power of bioassessments and aid in identifying the causal nature of the observed impacts. However, low macroinvertebrate abundance also difficult the use of multi-variate statistical treatments to detect ecological changes.

Although we did not find water acidification or high concentrations of dissolved metals in the sample system, the potential release of metals and acidity cannot be disregarded, because there are previous studies running different analysis that could effectively track it and link it to deleterious effects in the biota (e.g. Alvarenga et al., 2012;). Moreover, there are development plans (tourism) for the area and human health issues may then apply. Apart from this concerning human hazard, the environmental rehabilitation of mining areas during their post-mining phase is essential to keep the good ecological integrity of lotic systems and to ensure the continuity of terrestrial and aquatic species and *habitats*. In 2003, the process of environmental recovery of S. Domingos mine was started by a public company (EDM). Main activities consisted in the development of a hydrological and hydrogeological study of the open pit and mining complex; in the inspection of the dams; in the seal; in signalling and setting up observation points around the open pit and in the rehabilitation of the old railway station building of Pomarão. These activities ceased in 2005, and contributed only to mitigate security risks inherent to touristic activity in the mining complex, which was previously degraded and unstable (EDM, 2014). Under the Operational Programme for Sustainability and Efficiency in Use of Resources 2014-2020, development in environmental recovery works is expected (EDM, 2016). Thus, the investment in redevelopment projects in these areas will expectedly allow the definition of better environmental protection policies locally.

References

Adeniyi, A.A., Yusuf, K.A., Okedeyi, O.O. (2008). Assessment of the exposure of two fish species to metals pollution in the Ogun river catchments, Ketu, Lagos, Nigeria. Environ. Monit. Assess., 137, 451–458.

Akcil, A., & Koldas, S. (2006). Acid mine drainage (AMD): causes, treatment and case studies. *Journal of Cleaner Production*, 14, 1139–1145.

Alba-Tercedor, J., & Sánchez-Ortega, A. (1988). Um método rápido y simple para evaluar la calidad de las águas corrientes basado em el de Hellawell (1978). *Limnetica*, 4, 51-56.

Alvarenga, P., Palma, P., Varennes, A., & Cunha-Queda, A.C. (2012). A contribution towards the risk assessment of soils from the São Domingos Mine (Portugal): Chemical, microbial and ecotoxicological indicators. *Environmental Pollution*, 161, 50-56.

Álvarez-Valero, A.M., Pérez-López, R., Matos, J., Capitán, M., Nieto, J., Sáez, R., … Caraballo, M. (2008). Potential environmental impact at São Domingos mining district (Iberian Pyrite Belt, SW Iberian Peninsula): evidence from a chemical and mineralogical characterization. *Environmental Geology*, 55, 1797-1809.

APA (Associação Portuguesa do Ambiente) (2016). Boletim de precipitação. SNIRH (Sistema Nacional de Informação de Recursos Hídricos). Retrieved from http:// snirh. pt/ snirh/_ dadossintese/ precipitacao/ mapas_ resumo/entrada.php?periodo=2000%2F2015&mes=-1&ano= 2005 & tp _ mapa= mensal

APHA (1999). *Standard Methods for the Examination of Water and Wastewater*. Washington: American Public Health Association.

Bady, P., Dolédec, S., Fesl, C., Gayfraud, S., Bacchi, M. & Scholl, F. (2005). Use of invertebrate traits for the biomonitoring of European large rivers: the effects of sampling effort on genus richness and functional diversity. *Freshwater Biology*, 50, 159–173.

Barbour, M.T., Gerritsen, J., Snyder, B.D., & Stribling, J.B. (1999) *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish*. Washington: Environmental Protection Agency; Office of Water.

Bryan, C.G., Hallberg K. B., & Johnson, D.B. (2006). Mobilisation of metals in mineral tailings at the abandoned São Domingos copper mine (Portugal) by indigenous acidophilic bacteria. *Hydrometallurgy*, 83, 184-194.

Byrne, P., Reid, I., & Wood, P.J. (2013). Stormflow hydrochemistry of a river draining an abandoned metal mine: the Afon Twymyn, central Wales. *Environmental Monitoring and Assessment*, 185, 2817–2832.

Carlisle, D.M., & Clements, W.H. (1999). Sensitivity and variability of metrics used in biological assessments of running waters. *Environmental Toxicology and Chemistry*, 18, 285–291.

Chapman, D.V., & Jackson, J. (1996). Biological monitoring. *In* ‘Water Quality Monitoring – A Practical Guide to the Design and Implementation of Freshwater Quality Studies and Monitoring Programmes’ (Eds J. Bartram & R. Ballance.), pp. 263–302. (Published on behalf of UNESCO, WHO and UNEP by E&FN Spon, London, UK.).

Clements, W.H., & Kiffney, P.M. (1995). The influence of elevation on benthic community responses to heavy metals in Rocky Mountain streams. *Canadian Journal of Fisheries and Aquatic Sciences*, 52, 1966-1977.

Costa, M.C., & Duarte, J.C. (2005). Bioremediation of acid mine drainage using acidic soil and organic wastes for promoting sulphate-reducing bacteria activity on a column reactor. *Water, Air and Soil Pollution*, 165, 325-345.

Dahl, J., Johnson, R.K., & Sandin, L. (2004). Detection of organic pollution of streams in southern Sweden using benthic macroinvertebrates. *Hydrobiologia*, 516, 161–172.

De Bisthoven, J.L., Gerhardt, A., & Soares, A.M.V.M. (2004). Effects of acid mine drainage on Chironomus (Diptera, Chironomidae) measured with the multispecies freshwater biomonitor. *Environmental Toxicology and Chemistry*, 23, 1123-1128.

DeNicola, D.M., & Stapleton, M.G. (2002). Impact of acid mine drainage on benthic communities in streams: the relative roles of substratum vs. aqueous effects. *Environmental Pollution*, 119, 303-315.

Diaz, R.J., Solan, M., & Valente, R.M. (2004). A review of approaches for classifying benthic habitats and evaluating habitat quality. *Journal of Environmental Management*, 73, 165-181.

EDM (2014). Estudo hidrogeológico e de estabilidade de barragens, projectos e obras na área mineira de São Domingos. Retrieved from http://www.edm.pt/html/proj_saodomingos.htm.

EDM (2016). Estudos, projetos e obras previstos. Retrieved from http://www.edm.pt/html/projprev.htm.

El Khalil, H., El Hamiani, O., Bitton, G., Ouazzani, N., & Boularbah, A. (2008). Heavy metal contamination from mining sites in South Morocco: Monitoring metal content and toxicity of soil runoff and groundwater. *Environmental Monitoring and Assessment*, 136,147–160.

España, S.J., Pamo, E.L., Santofimia, E., Adurive, O., Reyes, J., & Barettino D. (2005). Acid mine drainage in the Iberian Pyrite Belt (Odiel river watershed, Huelva, SW Spain): Geochemistry, mineralogy and environmental implications. *Applied Geochemistry*, 20, 1320-1356.

EU (2000). Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *Official Journal of the European Communities*. L 327: 1-72.

Feld, C.K. (2004). Identification and measure of hydromorphological degradation in central European lowland streams. *Hydrobiologia*, 516, 55 69–90.

Gomes, M.E.P., Antunes, I.M.H.R., Silva, P.B., Neiva, A.M.R., & Pacheco, F.A.L. (2010). Geochemistry of waters associated with the old mine workings at Fonte Santa (NE of Portugal). *Journal of Geochemical Exploration*, 105, 153-165.

Gotelli, N., & Colwell, R. (2001). Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters*, 4, 379-391.

Gray, N.F. (1996). A substrate classification index for the visual assessment of the impact of acid mine drainage in lotic systems. *Water Research*, 30, 1551–1554.

Gray, N.F. (1997). Environmental impact and remediation of acid mine drainage: a management problem. *Environmental Geology*, 30, 62–71.

Gray, N.F. (1998). Acid mine drainage composition and the implications for its impact on lotic systems. *Water Research*, 32, 2122–2134.

Guérit, I., Bocquene, G., James, A., Thybaud, E., & Minier, C. (2008). Environmental risk assessment: A critical approach of the European TGD in an *in situ* application. *Ecotoxicology an Environmental Safety*, 71, 291-300.

Hering, D., Buffagni, A., Moog, O., Sandin, L., Sommerhäuser, M., Stubauer, I. … & Zahrádková, S. (2003). The development of a system to assess the ecological quality of streams based on macroinvertebrates – Design of the sampling programme within the AQEM project. *International Review of Hydrobiology*, 88, 345–361.

Hickey, C. W., & Clements, W. H. (1998). Effects of heavy metals on benthic macroinvertebrate communities in New Zealand streams. *Environmental Toxicology and* Chemistry, 17, 2338–2346.

Hickey, C.W., & Golding, L.A. (2002). Response of macroinvertebrates to copper and zinc in a stream mesocosm. *Environmental Toxicology and Chemistry*, 21, 1854-1863.

Hoiland, W.K., Rabe, F.W., & Biggam, R.C. (1994). Recovery of macroinvertebrate communities from metal pollution in the South Fork and mainstem of the Coeur d'Alene River, Idaho. *Water Environment Research*, 66, 84–88.

Hudson-Edwards, K.A., Jamieson, H.E., & Lottermoser, B.G. (2011). Mine wastes: Past, present, future. *Elements*, 7, 375-379.

INAG, (2008). *Manual para a avaliação biológica da qualidade da água em sistemas fluviais segundo a Diretiva Quadro da Água. Protocolo de amostragem e análise para os macroinvertebrados bentónicos*. Lisboa: Ministério do Ambiente, Ordenamento do Território e do Desenvolvimento Regional. Instituto da Água.

INAG, (2009). *Critérios para a classificação das massas de água superficiais – rios e albufeiras*. Lisboa: Ministério do Ambiente, Ordenamento do Território e do Desenvolvimento Regional. Instituto da Água.

ISO (2005). ISO Standard DIN EN ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories. International Organization for Standardization, Geneva, Switzerland.

Iwasaki, Y., Kagaya, T., Miyamoto, K., & Matsuda, H. (2009). Effects of heavy metals on riverine benthic macroinvertebrate assemblages with reference to potential food availability for drift-feeding fishes. *Environmental Toxicology and Chemistry*, 28, 354–363.

Iwasaki, Y., & Ormerod, S. (2012). Estimating safe concentrations of trace metals from inter-continental field data on river macroinvertebrates. *Environmental Pollution*, 166, 182-186.

Langmuir, D. (1997). Aqueous Environmental Geochemistry. New Jersey: Prentice Hall.

Lopes, I., Gonçalves, F., Soares, A.M.V.M., & Ribeiro, R. (1999). Discriminating the ecotoxicity due to metals and to low pH in acid mine drainage. *Ecotoxicology Environmental Safety*, 44, 207–214.

Lorenz, A., Hering, D., Feld, C.K., & Rolauffs, P. (2004). A new method for assessing the impact of hydromorphological degradation on the macroinvertebrate fauna of five German stream types. *Hydrobiologia*, 516, 107–127.

Lu, Y., & Allen, H. (2001). Partitioning of copper onto suspended particulate matter in river waters. *The Science of the Total Environment*, 277, 119-132.

Macan, T.T. (1959). *A guide to freshwater invertebrate animals*. London: Longman Group Limited.

Malmqvist, B., & Hoffsten, P.O. (1999). Influence of drainage from old mine deposits on benthic macroinvertebrate communities in central Swedish streams. *Water Research*, 33, 2415-2423.

Malmqvist, B., & Rundle, S. (2002). Threats to the running water ecosystems of the world. Environmental Conservation, 29, 134–153.

MAOT (Ministério do Ambiente e Ordenamento do Território) (2001). *Plano de Bacia Hidrográfica do Rio Guadiana – Relatório Final Partes I, II e III*. Instituto da Água. Ministério do Ambiente e Ordenamento do Território, 364 pp.

Maret, T.R., Cain, D.J., MacCoy, D.E., & Short, T.M. (2003). Response of benthic invertebrate assemblages to metal exposure and bioaccumulation associated with hard-rock mining in northwestern streams, USA. *Journal of the North American Benthological Society*, 22, 598–620.

Matos, J., Soares, S., & Cardoso, C. (2006). Caracterização geológica-geotécnica da corta da mina de S. Domingos, FPI. Actas do X *Congresso Nacional de Geotecnia*, pp. 741-752.

Mebane, C.A. (2001). Testing bioassessment metrics: macroinvertebrate, sculpin, and salmonid responses to stream habitat, sediment, and metals. *Environmental Monitoring and Assessment*, 67, 293–322.

MINEO (2003). Assessing and monitoring the environmental impact of mining activities in Europe using advanced earth observation techniques. Final Report – Section 6: Detailed Report.

Oberholster, P.J., Genthe, B., Hobbs, P., Cheng, P.H., Klerk, A.R., & Botha, A.M. (2013). An ecotoxicological screening tool to prioritise acid mine drainage impacted streams for future restoration. *Environmental Pollution*, 176, 244-253.

Oliveira, J.M.S., Farinha, J., Matos, J.X., Ávila, P., Rosa, C., Machado, M.J.C., … Leite, M.R.M. (2002). Diagnóstico Ambiental das Principais Áreas Mineiras Degradadas do País. *Boletim de Minas - Instituto Geológico e Mineiro (IGM)*, 39, 67-85.

Pattée, E., & Gourbault, N. (1981). *Turbellaries triclades paludicoles (Planaires d'eau douce). Introduction pratique a la systematique des organismes des eaux continentales françaises.* Paris: Association Française de Limnologie.

Pereira, A.M.M., Soares, A.M.V.M., Gonçalves, F., & Ribeiro, R. (1999). Test chambers and test procedures for in situ toxicity testing with zooplankton. *Environmental Toxicology and Chemistry*, 18 (9), 1956-1964.

Pereira, A.M.M., Soares, A.M.V.M., Gonçalves, F., & Ribeiro, R. (2000). Water-column, sediment, and *in situ* chronic bioassays with cladocerans. *Ecotoxicology and Environmental Safety*, 47, 27-38.

Pereira, R., Ribeiro, R., & Gonçalves, F. (2004). Plan for an integrated human and environmental risk assessment in the S. Domingos mine area (Portugal). *Human and Ecological Risk Assessment*, 10, 543-578.

Pérez-López, R., Álvarez-Valero, A.M., Nieto, J.M., Sáez, R., & Matos, J.X. (2008). Use of sequential extraction procedure for assessing the environmental impact at regional scale of the São Domingos Mine (Iberian Pyrite Belt). *Applied Geochemistry*, 23, 3452-3463.

Pérez-López, R., Delgado, J., Nieto, J.M., & Márquez-Gárcia, B. (2010). Rare earth element geochemistry of sulphide weathering in the São Domingos mine area (Iberian Pyrite Belt): A proxy for fluid-rock interaction and ancient mining pollution. *Chemical Geology*, 276, 29-40.

Quental, L., Bourguignon, A., Sousa, A.J., Batista, M.J., Brito, M.G., Tavares, T., … & Cottard F. (2002). MINEO Southern Europe environment test site - Contamination/impact mapping and modelling – Final report. Information Society Technologies, IST–1999-10337. 57 pp.

Richoux, P. (1982). *Coleopteres aquatiques (genres: adultes et larves). Introduction pratique a la systematique des organismes des eaux continentales françaises.* Lyon: Bulletin de la Societé Linnéenne de Lyon.

Rodríguez-Jordá, M.P., Garrido, F., & García-González, M.T. (2012). Effect of the addition of industrial by-products on Cu, Zn, Pb and As leachability in a mine sediment. *Journal of Hazardous Materials*, 213-214, 46-54.

Rosenberg, D.M., & Resh, V.H. (1993). *Freshwater Biomonitoring Macroinvertebrates*. New York: Chapman & Hall.

Sandin, L., & Hering, D. (2004). Comparing macroinvertebrate indices to detect organic pollution across Europe: a contribution to the EC Water Framework Directive intercalibration. *Hydrobiologia*, 516, 55–68.

Santos, E.S., Abreu, M.M., Nabais, C., & Magalhães, M.C.F. (2012). Trace element distribution in soils developed on gossan mine wastes and *Cistus ladanifer* L. tolerance and bioaccumulation. *Journal of Geochemical Exploration*, 123, 45-51.

Sardinha, I.D., Craveiro, D., & Milheira, S. (2013). A sustainability framework for redevelopment of rural brownfields: stakeholder participation at São Domingos mine, Portugal. *Journal of Cleaner Production*, 57, 200-208.

Serra, S., Coimbra, N., & Graça, M. (2009). *Invertebrados de Água Doce. Chave de Identificação das Principais Famílias*. Coimbra: Imprensa da Universidade de Coimbra.

Siwela, A.H., Nyathi, C.B., & Naik, Y.S. (2010). A comparison of metal levels and antioxidant enzymes in freshwater snails, *Lymnaea natalensis*, exposed to sediment and water collected from Wright Dam and Lower Mguza Dam. *Ecotoxicology and Environmental Safety*, 73, 1728-1732.

Sundermann, A., Lohse, S., Beck, L.A., & Haase, P. (2007). Key to the larval stages of aquatic true flies (Diptera), based on the operational taxa list for running waters in Germany. *Annales De Limnologie-International Journal of Limnology*, 43, 61-74.

Tachet, H., Bournaud, M., & Richoux, P. (1980). *Introduction à l'étude des macroinvertébrés des eaux douces*. Université Lyon: Association Française de Limnologie.

USEPA (2009). *National Recommended Water Qality Criteria.* Washington: Office of Water.

Varennes, A., Qu, G., Cordovil, C., & Goncalves, P. (2011). Soil quality indicators response to application of hydrophilic polymers to a soil from a sulphide mine. *Journal of Hazardous Materials*, 192, 1836-1841.

Vlek, H.E., Verdonschot, P.F.M., & Nijboer, R.C. (2004). Towards a multimetric index for the assessment of Dutch streams using benthic macroinvertebrates. *Hydrobiologia*, 516, 173–189.

Welsh, P.G., Lipton, J., Chapman, G.A., & Podrabsky, T.L. (2000). Relative importance of calcium and magnesium in hardness-based modification of copper toxicity. *Environmental Toxicology and Chemistry*, 6, 1624–1631.

Supplementary Material

Table S1. Macroinvertebrate abundance in each sampling site (Ephemeroptera - Ephemer).

		TG		TP		CP		ED		CD	
Class/Order	**Family**	2006	2014	2006	2014	2006	2014	2006	2014	2006	2014
Coleoptera	Gyrinidae				2						
Crustacea	Atyidae										184
Diptera	Ceratopogonidae		15			4			2	12	
Diptera	Chaoboridae	1									
Diptera	Chironomidae	117	173	344	42	72	8		42	49	52
Diptera	Simulidae		1						1		
Ephemer	Baetidae	2				5	3		1		14
Ephemer	Caenidae	344	208	213	36	8	21	1	122		4
Ephemer	Ephemeridae		3								
Ephemer	Leptophlebiidae					2					
Ephemer	Polymitarcidae					2					
Gastropoda	Physidae				1						
Heteroptera	Corixidae	10	23	2	42	4		158	9	75	3
Heteroptera	Hebridae									1	
Odonata	Platycnemididae										12
Oligochaeta	Lumbriculidae			5		7					
Oligochaeta	Tubificidae			55		56					
Trichoptera	Ecnomidae						1				
Trichoptera	Glossosomatidae				1						
Trichoptera	Limnephilidae						1				
Trichoptera	Psychomidae				1						
Trichoptera	Sericostomatidae					1					

CAPÍTULO IV

Are biological invasions invisible to ecological quality metrics? A case study with the Asian clam in a semi-artificial drainage catchment

Abstract

The Asian clam *Corbicula fluminea* is considered one of the most successful invasive species in ecosystems, where it promotes severe ecological impacts. Still, the role imposed by this and other invasive species in constraining water quality *sensu* Water Framework Directive (WFD) has barely been addressed. This was discussed in the present study, following on a detailed inspection of the environmental constraints to *C. fluminea* dispersal in a semi-natural drainage catchment, which constitutes an interconnected channel network, in the northern centre of Portugal. A snap-shot sampling widely covering the drainage catchment (40 sampling sites, including invaded and non-invaded sites) was carried out to thoroughly characterise *C. fluminea* populations and, in parallel, the water column, sediment and hydromorphology. Macroinvertebrate communities were also sampled, allowing the bioassessment of the water quality variation throughout the system *sensu* WFD. The results highlight the large ecological competence of the clam. Despite the intrinsic connectivity of the study area, significant environmental gradients could be found in the water column and in channel hydromorphology that were weakly correlated with *C. fluminea* density distribution and importantly, do not provide support for the absence of clams from non-invaded sites. On the other hand, sites within the study area were essentially homogeneous as to sediment type and organic load; the related clam preferences as defined in the literature were not met but this did not prevent the establishment of the clams. Also, the distribution of ecological quality ratios calculated for all sites based on the benthic macroinvertebrate communities was largely unaffected by the clams distribution. However, community structure analysis suggested that *C. fluminea* favours some functional feeding groups. These converse observations seem to indicate that the bioassessment *sensu* WFD may disregard important drivers of ecological change in freshwater ecosystems.

Key Words: Asia clam, *Corbicula fluminea*, invasion, Water Framework Directive (WFD), benthic macroinvertebrates.

Introduction

The widespread degradation of freshwater ecosystems and the need for its systematic assessment is well-documented, triggering the endorsement of key legislative acts such as the Water Framework Directive (WFD; Directive 2000/60/EC). A key innovation in this context is the valuation of the ecological assessment of aquatic systems, adding to the traditional physical and chemical lines of evidence (Hering et al., 2003). The ecological status of freshwaters can be challenged by numerous agents and the spread of non-indigenous invasive species is reportedly a major driver of environmental change. These nuisances have actually been identified as one of the major threats to aquatic ecosystems, causing biodiversity losses and adverse environmental impacts (Pimentel, Zuniga, & Morrison, 2005). Bivalve invaders, in particular, have major abilities as physical ecosystem engineers by creating, modifying and maintaining new habitats, ultimately modulating the overall availability of resources (e.g., Sousa, Gutiérrez, & Aldridge, 2009).

The Asian clam *Corbicula fluminea* fits globally this picture, being currently considered one of the 100 worst invasive species in Europe (DAISIE, 2008). It is an opportunistic freshwater bivalve that spread from its native distribution range in Southeast Asia to Europe, North and South America over the last century (Araujo, Moreno, & Ramos, 1993; Phelps, 1994; Darrigran, 2002). Its invasive success stems from a rapid, productive and efficient life-cycle (Aldridge & McMahon, 1978; reviewed in McMahon, 2002), a reproductive system with hermaphroditism associated to optional self-fertilisation (Britton & Morton, 1982; Kraemer & Galloway, 1986), high ecological competence, close association with human activities, wide genetic variability and phenotypic plasticity, and generalist filter-feeding habits (Sousa, Antunes & Guilhermino, 2008; Sousa, Novais, Costa, & Strayer, 2014).

Negative ecological impacts of *C. fluminea* have been frequently evidenced and argued to ultimately lead to important alterations in ecosystem functioning. As reviewed by Sousa et al. (2008), the invasion by this clam can affect submerged vegetation, phytoplankton, zooplankton, and higher trophic levels; also, dense populations have been shown to link to the decline of native bivalves, mostly on the basis of the referred life-history and ecological skills (Strayer, 1999; Vaughn & Hakenkamp, 2001; Darrigran, 2002; Sousa et al., 2008; Sousa, Ilarri, Souza, Antunes, & Guilhermino, 2011). Massive die-offs of the Asian clam constitute a remarkable nutrient input in the system that provides the scope for a rapid degradation of water quality (Cherry, Scheller, Cooper, & Bidwell, 2005; Cooper, Bidwell, & Cherry, 2005). Positive effects of the Asian clam have also been pointed out. Some benthic species can benefit from the enrichment in empty shells (Ilarri et al., 2012; 2014), organic matter or nutrients released from the sediments to the water column (reviewed in Vaughn & Hakenkamp, 2001). The filtration ability of these clams contributes to significant increases in water clarity, controlling phytoplankton, benefiting the growth of submerged vegetation and benthic algae (e.g., Phelps, 1994). The remarkable increase of secondary production promoted by dense populations of *C. fluminea* (Marsh, 1985; Sousa, Nogueira, Gaspar, Antunes, & Guilhermino, 2008d) as an overall effect *per se*, plus the interplay of the above effects over particular ecosystem compartments/components, drive important changes that are likely to reflect in the ecosystem functioning and ecological status. The biofouling activity of the Asian clam is the other side of the coin, and the most immediate face of its economic impact. Dense populations can be drawn from invaded waterbodies and accumulate in low-flow areas (e.g. pipes, intermediate reservoirs, filters) within freshwater-dependent industries or irrigation settings, often translating into severe economic losses (Jenner, Whitehouse, Taylor, & Khalanski, 1998; Pimentel et al., 2005; Mackie & Claudi, 2010; Rosa et al., 2011).

Given the impacts of the Asian clam, the proper establishment of management practices is of critical importance. Furthermore, since dispersal into new habitats is a determinant stage in the invasion process (Davis, 2009), a robust understanding of the mechanisms involved and the ecological preferences of the pest is a key asset for its adequate management (Leung et al., 2002; Belz, Darrigran, Netto, Boeger, & Ribeiro, 2012; Kappes & Haase, 2012; Hoyer, Schladow, & Rueda, 2015). The rapid spread of *C. fluminea* involves human vectors (Britton, 1982; Brancotte & Vincent, 2002; Karatayev et al., 2007; Minchin, 2014), and passive transport by waterfowls (Britton, 1982) or fish (Cantanhêde, Hahn, Gubiani, & Fugi, 2008; Gatlin, Shoup, & Long, 2013). However, the major means of local dispersal has been assumed to be downstream transport with the water flow of juveniles and eventually upstream (Voelz, McArthur, & Rader, 1998; Mouthon, 2003; Hoyer, Schladow, & Rueda, 2015), assisted by their enhanced flotation capabilities (Prezant & Chalermwat, 1984).

The interconnectivity of drainage catchments provides favourable conditions for the spread of the clam (Lucy, Karatayev, & Burlakova, 2012), but there are only a few studies addressing its dispersal dynamics in such systems (e.g., Lucy et al., 2012; Minchin, 2014). Thus, some uncertainties are still unresolved regarding facilitating factors and obstacles. For example, while the unique ecological conditions of these interconnected systems favour the establishment and growth of threatened sphaeriids (Watson & Ormerod, 2005), vegetated wetland waterways were shown to act as a barrier for the downstream dispersal of the planktonic larvae of the zebra mussel *Dreissena polymorpha* (Bodamer & Bossenbroek, 2008); this may happen with benthic *C. fluminea* juveniles. Furthermore, water level control is frequently necessary in these systems (Herzon & Helenius, 2008) and low-head dams or dikes are used for these purposes, thus posing additional challenges to dispersal. Also, as summarised in Gangloff, Hartfield, Werneke, & Feminella (2011), physical effects of these structures include warming up of the retained water or overall changes in the hydrological regime and substrate type, with consequent habitat reworking.

Observations in routine field campaigns over a drainage ditch system (see Methods and Rosa et al., 2014) motivated the present study. This drainage system is highly heterogeneous, with parallel and intercommunicating channels and ditches facilitating the spread of clams, as well as small dykes (for water level control) that can difficult their dispersal. In fact, Asian clam abundance is highly heterogeneous throughout the system and a few canals are still free from this invader. This provides a great opportunity to explore the distribution pattern of the pest, as well as the variables that best explain its spread and density, as well as its potential impacts in benthic communities. Furthermore, drainage catchments structurally resemble irrigation facilities susceptible to the fouling activity and consequent economic damage by the bivalve (Ingram, 1959; Prokopovich &

Hebert, 1965; Mackie & Claudi, 2010; Rosa et al., 2011). Because of this resemblance, ecological data can be useful to improve preventive and control attempts in irrigation facilities already infested or at risk of infestation. In this way, this study addressed i) *C. fluminea* spread patterns and establishment constraints in such an ecologically significant system. To do so, a snap-shot sampling widely covering the drainage catchment was established to thoroughly characterise the water column and the benthic compartments, as well as the hydromorphology of each site. This also allowed the bioassessment of the water quality variation throughout the system on the basis of the macroinvertebrate assemblages, aiming to assess ii) whether the Asian clam can be related to changes in benthic communities, and consequently (iii) if such putative impacts translate into changes in the ecological status classification *sensu* WFD.

Materials and Methods

Study area and sampling strategy

This study was carried out in a semi-natural drainage catchment in the littoral centre of Portugal (Figure 1), included in the hydrographic basin of the Vouga river, and crossing the municipalities of Aveiro, Mira and Cantanhede. This is essentially a network of interconnected main channels and ditches collecting the water and deriving into small tributary brooks, much resembling the structure of agricultural irrigation settings. A series of sequential dikes (mechanically operated low-head dams) control the water level throughout and avoid flooding or excessive saline intrusion from the downstream Ria de Aveiro lagoon (Aveiro, Portugal). This system has actually been used for the irrigation of local agriculture fields; it was in the past an important resource in water-powered milling and, although all the abundant milling facilities are currently deactivated, almost all the associated dikes were kept active for water-level control purposes.

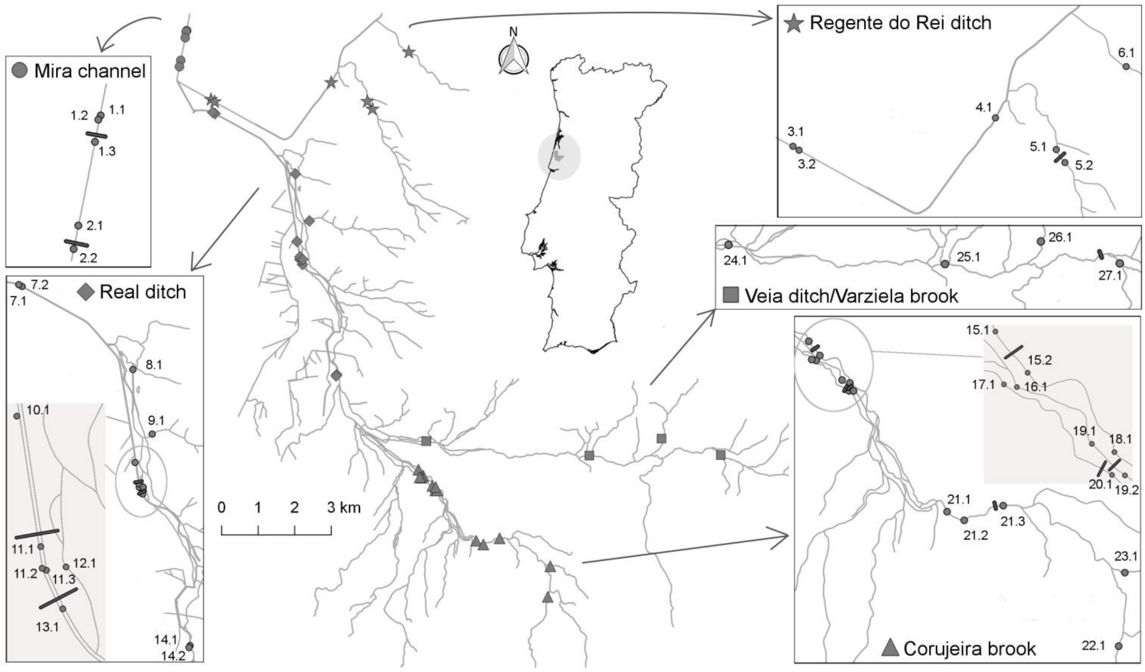


Fig. 1. Geographic location of the study area, which was artificially divided into 5 systems, all separately zoomed in the left- and right-hand panels of the figure: Mira channel, grouping 5 sites (1.1 – 2.2); Regente do Rei ditch, grouping 6 sites (3.1 – 6.1); Real ditch, grouping 12 sites (7.1 – 14.2); Corujeira brook, grouping 13 sites (15.1 – 22.1); Veia ditch and Varziela brook, grouping 4 sites (24.1 – 27.1). Details within Real ditch and Corujeira brook were further zoomed in grey areas to allow a proper picture of all sampling sites. Site numbering followed an ascending order from downstream to upstream, and different decimals for a given integer site designation indicate either recognised habitat variants within the same location or paired/triplet sampling near a dike (e.g. 2.2 was located at the head of the dike and 2.1 downstream the dike). Sites are represented by symbols and dikes are represented by bacillary bars.

The Asian clam has been present in the drainage catchment for more than a decade as informed by locals, and its seasonal dynamics has been scrutinised in detail by Rosa et al. (2014). In the present study, a snap-shot sampling strategy was employed (see e.g. Grayson, Gippel, Finlayson, & Hart, 1997) by directing research to the spatial variation within the drainage area, primarily addressing dispersal patterns and habitat preferences of the Asian clam in an instant in time. Five semi-lotic systems with different degrees of connectivity were sampled within the study area during late spring 2014 (Figure 1): Mira channel (MC); Regente do Rei ditch (RR); Real ditch (RD); Corujeira brook (CB); Veia ditch/Varziela brook (VV). Sampling sites were defined within each sub-catchments (main course and tributaries) taking into account site heterogeneity due to variation in channel morphology and the occurrence of dikes; exact location of sampling sites was frequently adjusted considering accessibility. Following a catchment-wide approach, a total of 40 sampling sites (Figure 1) were comprehensively characterised considering the following components (see below for details): i) water physico-chemistry; ii) sediment organic burden and particle size distribution; iii) complementary habitat characterisation (hydromorphology); iv) benthic macoinvertebrate community structure; v) ecological status (based on macroinvertebrate diversity and presence of sensitive *vs*. tolerant groups); vi) Asian clam density and size structure.

Water analysis

Water temperature ('Temp'), pH, dissolved oxygen ('Oxi') and conductivity ('Cond') were recorded *in situ* using a multiparameter field probe (WTW-Multi3430). A 3-L water sample was collected and transported in the dark at 4ºC to the laboratory for immediate characterisation. Here, each sample was vacuum filtered through glass microfiber filters (1.2 µm pore size; 47 mm ø) and the residue was used to quantify total suspended solids ('TSS', mg L^{-1}; APHA, 1995) and, independently, Chl *a* content ('Chl*a*', µg L^{-1}; Lorenzen, 1967). The filtrate was used to quantify coloured dissolved organic carbon ('CDOC', m^{-1}; Williamson, Morris, Pace, & Olson, 1999), as well as calcium, alkalinity and hardness ('Ca', 'Alkal', 'Hard'; mg L^{-1}) through colorimetric methods (APHA, 1995). Turbidity was indirectly determined through the absorption coefficient at 450 nm (colour) of the raw water samples ('Turb', m^{-1}; Brower, Zar, & von Ende, 1998). Aliquots of untreated samples were mineralised with potassium persulphate (Ebina, Tsutsui, & Shirai, 1983) for latter quantification of total phosphorous content through the tin(II) chloride method ('TP', mg P L^{-1}; APHA, 1995) and total nitrogen content through the cadmium reduction method ('TN', mg N L^{-1}; Lind, 1979).

Sediment analysis

Sediment samples from the top 5 cm (the *habitat* of the macroinvertebrate assemblage also sampled; see below) were directly collected into plastic bags for particle size analysis to avoid loss of fine particles; this procedure was carried out at 3-5 locations within the same site to achieve a composite sample (~500 g) reflecting small-scale patchiness. The samples were transported to the laboratory at 4ºC in the dark, and then preserved at -20ºC until further processing, which occurred within 3 weeks after collection. Prior to analysis, the samples were thawed at room temperature and roughly sorted to remove large organic debris, and they were then oven-dried (70ºC for 24 h) for further determination of loss-on-ignition (450ºC for 6 h) as a measure of organic matter content ('OM', % w/w; Kristensen & Andersen, 1987). The incinerated (organic-free) samples were used for the quantification of fines (% w/w silt and clay) through wet sieving (≤ 63 µm) with a sodium hexametaphosphate dispersant solution (Pope et al., 2000). The retained fraction (> 63 µm) was oven dried and weighed to the nearest 0.01 g; the following fractions were mechanically separated in a sieve shaker, and then weighed: > 4000 µm, 4000-2000 µm, 1000-2000 µm, 500-1000 µm, 250-500 µm, 125-250 µm, and 63-125 µm. All particle size fractions (including fines) were converted to the Wentworth and Krumbien phi scale, and the median, mode and quartile deviation ('PhiQD', which is a measure of particle sorting) were determined by graphical interpolation of the cumulative particle-size distribution (Pope et al., 2000).

Habitat characterisation and hydromorphology

Complementary *habitat* characterisation was carried out in all sites by monitoring abiotic and biotic parameters that could constrain or relate to the establishment of the clams. Many of these variables are commonly used in routine monitoring programmes as part of the hydromorphological characterisation of river stretches (e.g., river *habitat* survey; Raven, Dawson, & Everard, 1998). The following parameters were quantified: depth (m); channel and water width ('Cwid' and 'Wwid'; m); flow velocity ('Flow'; m s^{-1}), which was recorded as a mean of 3 independent measurements at each site with a flowmeter (Global water, FP101); type of macrophytes present (presence of emergent, floating or filamentous macrophytes; 'EmeM', 'FloM' or 'FilM') and presence of filamentous algae ('FilA') within the channel; continuity of riparian vegetation in the banks (scaled 1-4 considering right or left bank area; ‘RBrip’ and ‘LBrip’); shading (‘shade’, scaled 1-3 considering the covered channel area).

Macroinvertebrates sampling and processing

Macroinvertebrate sampling was made according to the proportional presence of microhabitats (Hering et al., 2003; INAG, 2008a), and benthic macroinvertebrates were collected by kick-sampling small transects (standard hand net with square frame of 0.30 x 0.30 m; 500 µm pore size), covering similar area and sampling effort (in time) across sites. Collected samples were placed in air-tight plastic containers and preserved with 80-90% ethanol (Barbour, Gerritsen, Snyder, & Stribling, 1999) for further analysis. In the laboratory, preserved benthic macroinvertebrate samples were washed through a 500-µm-mesh size sieve. Sorted organisms were counted and identified to the lowest practical taxonomical level, generally the family (or sub-family, when applicable) using general and taxon-specific identification keys (Hynes, 1993; Tachet, 2000; Wallace, Wallace, & Philipson, 2003; Edington & Hildrew, 2005; Sundermann, Lohse, Beck, & Haase, 2007; Serra, Coimbra, & Graça, 2009; Elliott & Humpesch, 2010; Pawley, Dobson, & Fletcher, 2011). A multivariate data matrix of taxon site abundance was produced after excluding *C. fluminea* records (Table S1), because the macroinvertebrate dataset was afterwards related to the clam presence and density (see Statistics). The following macroinvertebrate community metrics were calculated for each site, using the family as the taxonomical resolution level: total number of families (richness, *S*), diversity (Shannon’s *H’*) and equitability (Pielou’s *J’*). In parallel, three biotic indices – based on presence/absence of pollution-tolerant and sensitive taxa – were also calculated: i) EPT, i.e. the number of *taxa* belonging to orders Ephemeroptera, Plecoptera and Trichoptera; ii) IBMWP, the sum of pre-defined tolerance scores for all the taxa present in the sample – specifically adapted to monitor benthic macroinvertebrates in the Iberian Peninsula (Alba-Tercedor & Sánchez-Ortega, 1988; Jaímez-Cuéllar et al., 2002); and iii) IASPT, which is simply the average score per *taxon*, derived from IBMWP.

Determination of ecological status (WFD approach)

The macroinvertebrate community was used as an indicator of ecosystem health, and the ecological quality of each sample was determined as an Ecological Quality Ratio (EQR), an approach endorsed by European regulatory bodies via Water Framework Directive (WFD). EQRs express the level of deviation of the macroinvertebrate communities relatively to a pre-established reference situation defined by national agencies, after a regional intercalibration process (JRC, 2009; Munné & Prat, 2009). For this specific system, EQRs were derived from the multimetrix index $IPtI_S$ (South Invertebrate Portuguese Index; INAG, 2009; JRC, 2009):

$IPtI_S = 0.4 \times S + 0.2 \times EPT + 0.2 \times (IASPT - 2) + 0.2 \times log$ (sel. EPTCD + 1), where S, EPT, IASPT and *log* (sel. EPTCD +1) are community metrics and biotic indices that must be normalised (i.e. divided by their corresponding reference values) prior to calculation. The first three metrics have already been described above; log (sel. EPTCD + 1) is the logarithm (plus one) of the sum of abundances of organisms belonging to families Chloroperlidae, Nemouridae, Leuctridae, Leptophlebiidae, Ephemerellidae, Philopotamidae, Limnephilidae, Psychomyiidae, Sericostomatidae, Elmidae, Dryopidae, Athericidae. EQRs were calculated through a second normalisation step, by dividing the $IPtI_S$ of each sample with the corresponding reference value. Reference values for macroinvertebrate community metrics, biotic indices, and IPtIs were obtained from official guidance documents (INAG, 2009), considering the sampled waterbodies as a type L river – rivers from central littoral (INAG, 2008b). For this river typology, the following intervals were used for determining ecological status: “High”, if EQR > 0.74; “Good”, if 0.56 < EQR < 0.74; “Moderate”, if 0.37 < EQR < 0.56; “Poor”, if 0.19 < EQR < 0.37; and “Bad”, if EQR < 0.19 (INAG, 2009).

Asian clam sampling and processing

Asian clam populations were sampled with a Van Veen grab sampler (0.05 m^2), obtaining a composite sample of 0.25 m^2 in each site, by pooling the content of 5 grabs (3 grabs or 0.15 m^2 were sampled in sites with exceptionally high clam densities). The collected sediment was sieved with a 1-mm mesh size bag; the retained sample was carefully transferred into plastic bags for transportation and stored at -20ºC until further processing. The clams were sorted out from thawed samples, counted, and individual shell lengths were measured to the nearest 0.01 mm with a digital Vernier caliper. Clam abundance was expressed as density, i.e. the number of individuals per square meter (ind m^{-2}).

Statistics

In order to quantitatively address putative dispersal constraints of the Asian clam within the studied catchment, environmental data were compiled into multivariate matrices, separated into three subsets: i) water subset, compiling water physico-chemical parameters; ii) sediment subset, pooling records on organic load and particle size; iii) hydromorphology subset, summarising morphology of the channel and banks, flow velocity and presence of aquatic vegetation. Principal Component Analysis (PCA) was run to address spatial gradients within each environmental subset; centering and standardisation of the variables was applied to avoid scaling effects (Ter Braak, 1995), and biplots were used to interpret gradients using symmetrical scaling (Gabriel, 2002). Extracted gradients (PCA components) for each environmental subset were further analysed by exploring the PCA sample scores of the first two axes (which explain most of the variation in the data). One-way ANOVA on the sample scores was used to test whether sites invaded by *C. fluminea* differed from sites where the clams were not found, in terms of the site's water chemistry, sediment, or hydromorphological profiles. Correlation analysis was used to explore the association between PCA scores and the density of *C. fluminea* (*log*-transformed to improve linearity). Correlation was performed considering a matrix with all sites or only invaded sites; this was deemed necessary as a way to accommodate the possibility of the clams being absent due to dispersal constraints not related with water chemistry, sediment, or hydromorphology, which could cause potentially spurious correlations when all sites are considered.

A fourth subset of data was set to address further research questions of the present study, regarding the effects of the invasion by the Asian clam in water quality and benthic community structure. This biotic subset included the metrics calculated for the macroinvertebrate community (abundance, H', J', EPT, IBMWP, IASPT, and $IPtI_S$). A similar approach to the environmental subsets (see above) was used, with PCA being followed by ANOVA and correlation analysis on PCA sample scores. Given the overall acceptability (namely within the WFD) of EQRs as integrated indicators of the ecological status of waterbodies, a one-way ANOVA was also run to inspect for differences in EQR between invaded and non-invaded sites, and correlation analysis was used to relate EQR scores with *log* density of *C. fluminea*.

Detrended Correspondence Analysis (DCA) was run to analyse spatial gradients in macroinvertebrate community structure, using the untransformed matrix of species abundances. This technique assumes an underlying unimodal response model that better fits abundance data (Ter Braak, 1995). Follow-up one-way ANOVA and correlation analyses were run as above, relating the extracted gradients (in the form of DCA sample scores) to *C. fluminea* presence and density (either in all or only in invaded sites). As an additional exploratory effort to understand the putative effect of the clams over the macroinvertebrate communities, Canonical Correspondence Analysis (CCA) was run (Ter Braak, 1995) with the untransformed macroinvertebrate species matrix. Two data matrices were used as explanatory data: i) an environmental matrix pooling all variables from each environmental subset (sediment, water and hydromorphology; see above); ii) a clam matrix composed of a binomial variable expressing the presence (1) or absence (0) of clams and a continuous variable expressing clam density at each site (*log* density +1). Manual forward selection of environmental variables was carried out to reduce the environmental dataset to non-redundant and significant (Monte Carlo permutation tests, $P \leq 0.05$) variables. Using the variation partitioning technique described by Borcard, Legendre, & Drapeau (1992), CCA and partial CCA allowed ascribing the amount of variation of the macroinvertebrate dataset to the catchment's environmental framework or to clam presence and density (see other examples in Castro & Gonçalves, 2011; Vidal et al. 2014). As such, these procedures could clarify whether Asian clams could explain some variation in the macrobenthic communities, after removing (i.e., partialling out, *sensu* Ter Braak, 1988) confounding environmental influences.

CCA models were tested for significance ($P \leq 0.05$) using a Monte-Carlo unrestricted permutation test.

All multivariate analyses were run in CANOCO 4.5 (Scientia Ltd., UK); other statistics were applied using Minitab 16 (Minitab, Inc., USA).

Results

The records on the density of the Asian clam confirmed our empirical observations, showing clearly that the nuisance is not distributed homogeneously, despite the intrinsic connectivity of the study area (Figure 2). Whole sub-catchments RR and VV are still free from the invader, while the remaining sub-catchments show a remarkable variation in clam density, ranging between 4.1 clams m^{-2} in site 17.1 and 3076.9 clams m^{-2} in site 12.1. The absence of clams in sites 9.1 and 23.1 is noticeable taking into account that these sites are integrated in the highly invaded RD and CB sub-catchments, but both sites stand in small tributaries of the main channel. Overall, the size distribution of individuals reveals young populations in most sites, with larger size classes (> 20 mm shell length) being clearly less abundant. In the lower reaches of the drainage system (see MC and sites 7.1 and 7.2 in RD), the youngest individuals (i.e. smallest, with shell length below 5 mm) are the dominant size class of the population. The densities of very small individuals were always higher here than in the remaining upstream sites, where this size class is rare (it can only be found in sites 11.2, 12.1, 13.1, 16.1 and 19.1). Apart from this downstream distributional constraint for larger individuals, the overall clam distribution pattern suggests that dikes have no barrier effect in the spread of the clams.

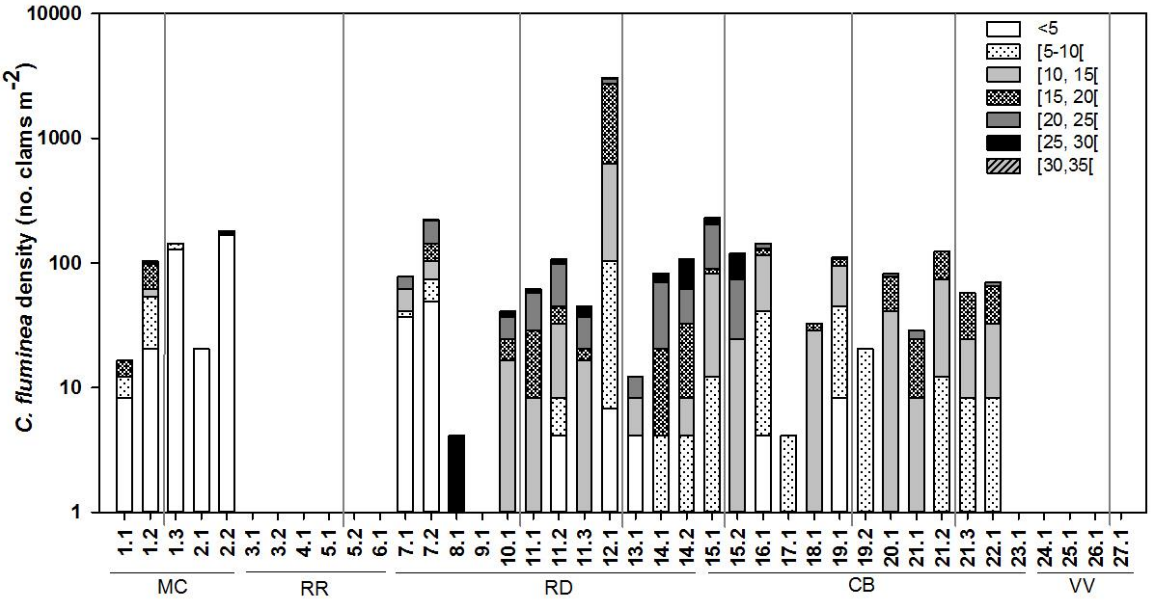


Fig. 2. Total density of *Corbicula fluminea* in the sampled sites. Stacked bars are informative of the population composition in terms of the individuals' shell length at each of the sampled sites, which are grouped over the category horizontal axis into five identifiable semi-lotic systems within the study area. MC stands for Mira channel and includes the most downstream sites; RR, RD and CB abbreviate Regente do Rei ditch, Real ditch and Corujeira brook, respectively; VV stands for Veia ditch and Varziela brook, which include the most upstream sites. The grey vertical lines indicate the relative position of the main dikes within the systems.

Distribution of C. fluminea across environmental gradients

The PCA biplots (Figure 3) provide a meaningful picture of the variation across sites according to each environmental subset - raw datasets are available in Tables S2-S4. In all cases, the variance explained by the first two components was always close to or above 50%.

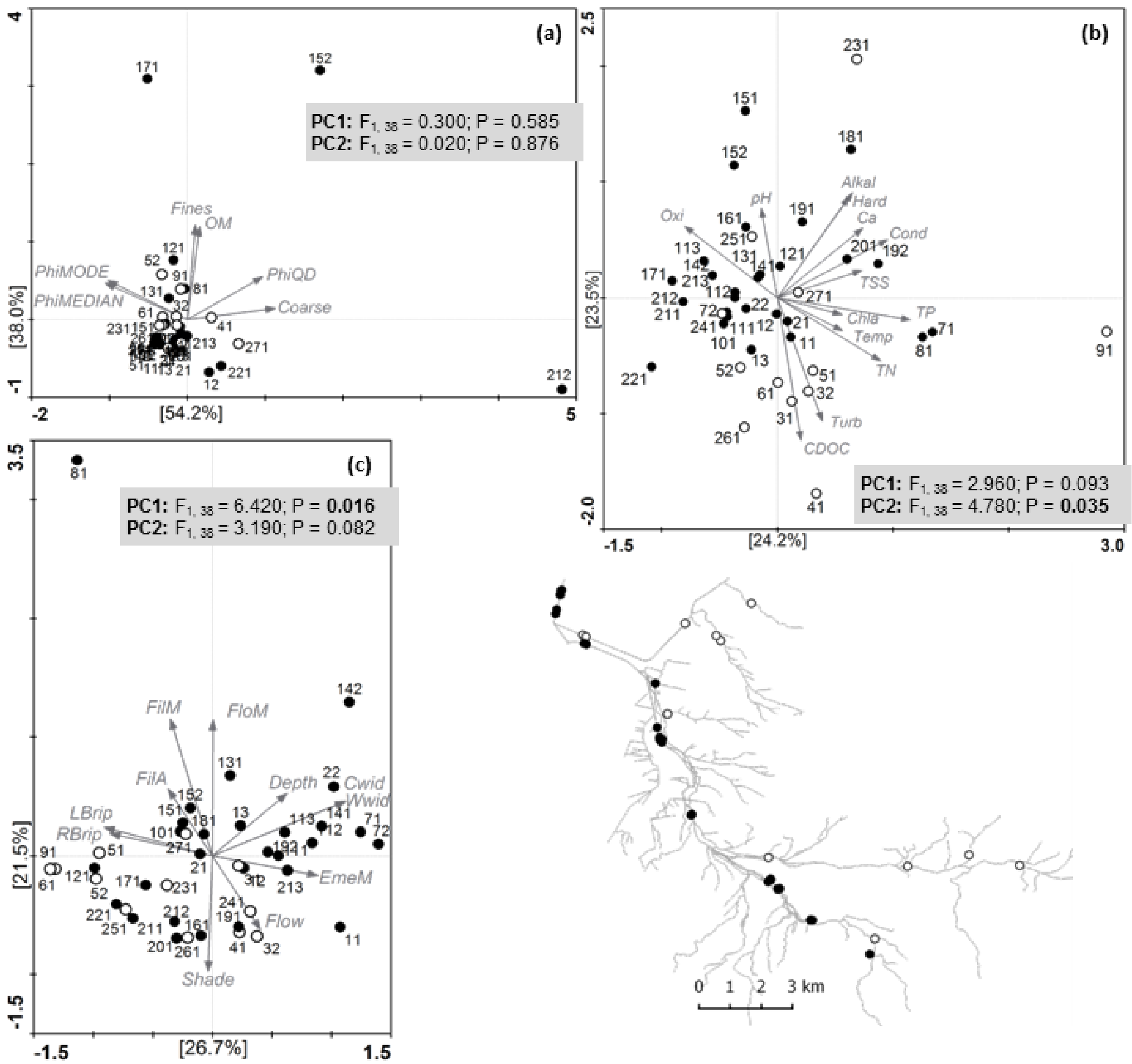


Fig. 3. Principal Component Analysis (PCA) biplots synthesizing the distribution of the sampled sites according to (a) the sediment subset; (b) the water subset; (c) the hydromorphology subset. The biplots, and the map with the distribution of the sampling sites added to the lower right corner of the figure to assist its interpretation, were post-edited to distinguish sites invaded (black circles) and non-invaded (white circles) by the Asian clam. The grey arrows represent the gradients given by each variable used in the analysis as labelled also in grey; labels are either self-explanatory or can be found clarified in the Methods section. For clarity purposes, the dots were excluded from the site's ID (see Figure 1 as a reference for the actual IDs), thus, for example, site 1.1 reads here as 11 while site 14.2 reads here as 142. The percentage of variance explained by PC1 (horizontal axis) and PC2 (vertical axis) as retrieved in each eigenanalysis was added to the corresponding axis in each diagram within square brackets. One-way ANOVA summaries distinguishing invaded and non-invaded sites were displayed in each biplot shadowed grey.

The sampling area was generally homogeneous in terms of sediment characteristics (Figure 3a): although PC1 and PC2 were associated with particle size structure and organic load, respectively, dense clustering of sample scores close to the origin was observed. Sediments were mostly composed by coarse- and medium-granule sand, with low particle sorting (phi QD), confirming the overall homogeneity of sediments as to particle size distribution. Sites 15.2, 17.1 and 21.2 (CB) were a noticeable exception to this trend, as inferred from their position in the diagram (Figure 3a). The first two had a high percentage of fines (11.6 and 16.5%) and organic matter content (5.6 and 4.2%); sites 15.2 and 21.2 were also distinctly different from the other sites given their high percentage of coarse (> 2 mm) particles (29% and 62%, respectively). No distinction between invaded and non-invaded sites was found for any of the extracted principal components (see ANOVA summary in Figure 3a), indicating that sediment characteristics explain poorly the distribution of Asian clams in the drainage area. Moreover, sediment characteristics also seem unimportant as a driver of clam density in sampled sites, given the absence of significant correlations between density records and PC1 or PC2 (Table 1).

Table 1. Summary of the correlation analyses attempted considering all sites or only the invaded sites, between *log* density of *C. fluminea* and the scores of the first two axis of the corresponding multivariate approach - PC1 and PC2 for the PCAs run with the environmental subsets and the biotic metrics; Axis 1 and Axis 2 for the DCA run considering the abundances of macroinvertebrate taxa. Significant correlations were highlighted bold.

	All sites		**Invaded sites**	
	Pearson coeff.	**F**	**Pearson coeff.**	**F**
Sediment PC1	0.145	0.371	0.144	0.464
Sediment PC2	-0.057	0.727	-0.170	0.388
Water PC1	-0.291	0.068	-0.163	0.408
Water PC2	0.355	**0.025**	0.211	0.282
Hydromorphology PC1	0.375	**0.017**	0.107	0.586
Hydromorphology PC2	0.116	0.475	-0.288	0.137
Biotic metrics PC1	0.036	0.824	-0.123	0.534
Biotic metrics PC2	-0.076	0.639	-0.044	0.824
Macroinvertebrates Axis 1	0.187	0.253	0.463	**0.015**
Macroinvertebrates Axis 2	0.104	0.529	-0.190	0.342

Unlike the sediment profile, the water physico-chemistry across sampled sites showed heterogeneity within the drainage system, with scores scattered according to mineralisation (conductivity, hardness) and organic load (TN, CDOC, turbidity) gradients (Figure 3b). A rough spatial grouping of sites according to the presence/absence of Asian clams is apparent in the PCA biplot (Figure 3b), and this was confirmed by significantly lower PC2 scores in non-invaded sites (see ANOVA summary in Figure 3b). This suggests that the clams generally establish themselves in sites with higher dissolved oxygen and/or higher pH and more mineralised waters. On the other hand, they are not present in more turbid and carbon-rich waters. Still, there are sites providing converse evidence of this, such as 23.1 (CB) or 25.1 (VV), where the clam was absent despite high oxygen levels and high mineralisation. Asian clam density is only partially dependent on water physico-chemistry, as indicated by the significant correlation between density and PC2 scores for all sites, which then became non-significant when only invaded sites were considered (Table 1).

The hydromorphology of the drainage system was also heterogeneous (Figure 3c), with a marked contrast between wider and deeper channels, where emergent macrophytes are present (sites located on the right side of biplot), as well as between narrower and shallower channels, where riparian coverage is denser (left side of diagram). Sites with higher filamentous and floating-leaved macrophyte cover (top side of the biplot) oppose sites where macrophytes were practically absent (bottom side), and where shading was higher (see opposing vertical gradients in Figure 3c). The first gradient was related to the distribution of the clams, as revealed by a significant distinction of PC1 scores between invaded and non-invaded sites; it also correlated significantly with *C. fluminea* density, but only when all sites were considered (ANOVA summary in Figure 3c; Table 1). A more in-depth analysis of the PCA biplot shows that, in fact, the invader occurs throughout the entire environmental gradient (see black circles in Figure 3c), but it is less frequent in shallower and narrower channels (bottom left side of the diagram).

Impact of C. fluminea *on ecological status and on the structure of benthic communities*

The biotic subset integrating information retrieved from the benthic macroinvertebrate communities (raw data in table S5) revealed an ecological quality gradient, with samples with higher scores in biotic indices on the left site of the diagram (Figure 4a). Also, but less important, abundant and uneven samples (top) contrasted with less abundant but more even communities in some sites (bottom). Despite this heterogeneity, the biotic metrics were poorly related to the distribution of the clams, as confirmed by the absence of significant differences between invaded and non-invaded sites, either considering PC1 or PC2 (ANOVA summary in Figure 4a), and by the lack of significant correlation between PC scores and clam density (Table 1). Also, no significant correlations between EQR scores and clam density were found, when considering all sites (r = -0.065; P = 0.691) or when restricting the dataset to invaded sites (r = 0.052; P = 0.793). The occurrence of Asian clams in sites with variable ecological quality (as indicated by macroinvertebrates) seems to contradict the hypothesis that the establishment of the invader can negatively impact ecosystem quality. In this sense, sites holding clam densities >100 individuals m^{-2} cover almost the whole range of EQR classifications (Poor-Good) (Figure 4b). Also, the ecological status of several non-invaded sites was classified as Bad (25.1 from VV) or Poor (3.1-5.1 from RR; 27.1 from VV), while a single non-invaded site (26.1 from VV) attained Good ecological status. This was reinforced by the fact that no significant differences in EQR scores were found between invaded and non-invaded sites (ANOVA: $F_{1, 38}$ = 0.37; P = 0.535).

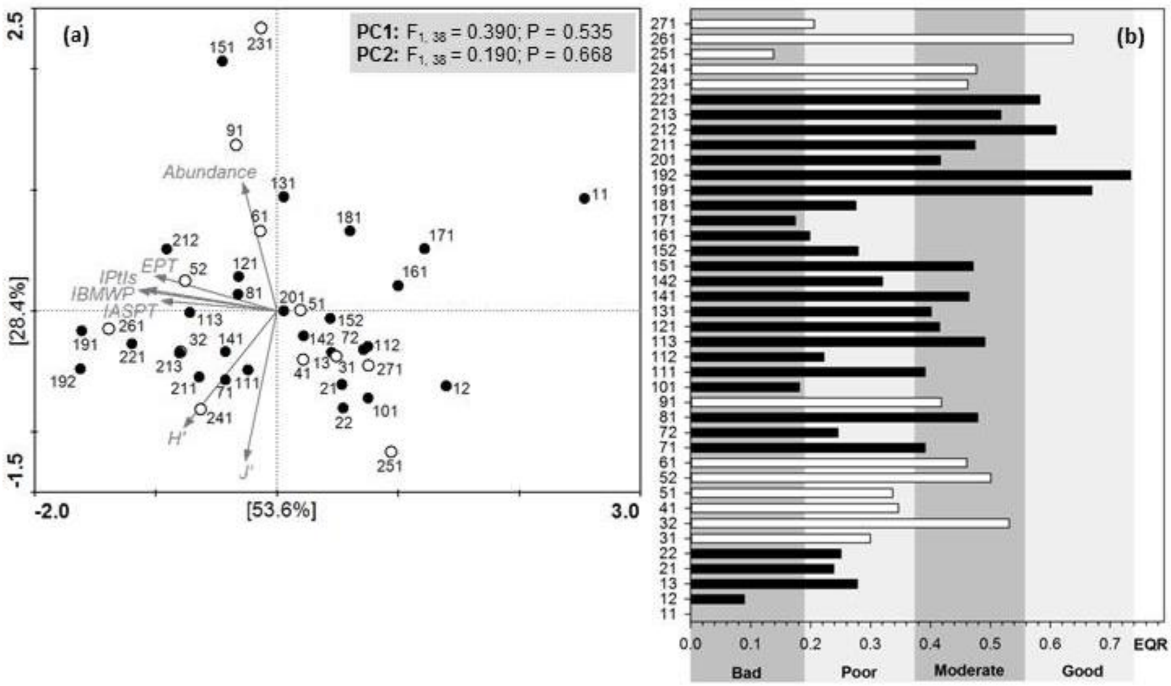


Fig. 4. PCA Biplot synthetizing the distribution of the sampled sites according to biotic metrics applied to the benthic macroinvertebrate community (a) and Ecological Quality Ratios calculated for all sampled sites (b). The plots were post-edited to distinguish sites invaded (black circles/bars) and non-invaded (white circles/bars) by the Asian clam. The grey arrows in (a) represent the gradients given by each variable used in the analysis, which were also labelled grey; labels in are either self-explanatory or can be found clarified in the Methods section. The percentage of variance explained by the PC1 (horizontal axis) and PC2 (vertical axis) was added to the diagram within square brackets, as well as was the one-way ANOVA summary distinguishing invaded and non-invaded sites shadowed grey. As in Figure 3, the dots were excluded from the site's ID for clarity purposes

Despite the observed spatial heterogeneity of the drainage area, in terms of its water quality, hydromorphology, and distribution of Asian clam, the macroinvertebrate community structure was fairly homogeneous throughout the whole system. This trend can be observed by the dense clustering of all sample scores in the DCA plot (Figure 5a), although the two DCA axes jointly explain 42.5% of the total variation of the data. Invaded and non-invaded sites did not differ significantly (see ANOVA summary in Figure 5) considering either axis 1 or axis 2 scores. This suggests that the clam invader does not constrain other benthic invertebrates within the sampling area. CCA corroborated this, by showing that environmental variables such as oxygen, TN, TP, flow, depth, Chl*a*, filamentous algae, SST, shade, channel width, phiQD and organic matter were, by decreasing order of relevance (and after a forward selection procedure), more important to explain the variation of the macroinvertebrate species composition than clam presence or density (Table 2). In fact, the environmental dataset was able to explain 65.5% of the variation of the macroinvertebrate community data, whilst clam contribution was negligible (2.9%) and non-significant. A small fraction of the data was explained by the intersection of both explanatory datasets (7.1%) and 24.5% of the total variation remained unexplained.

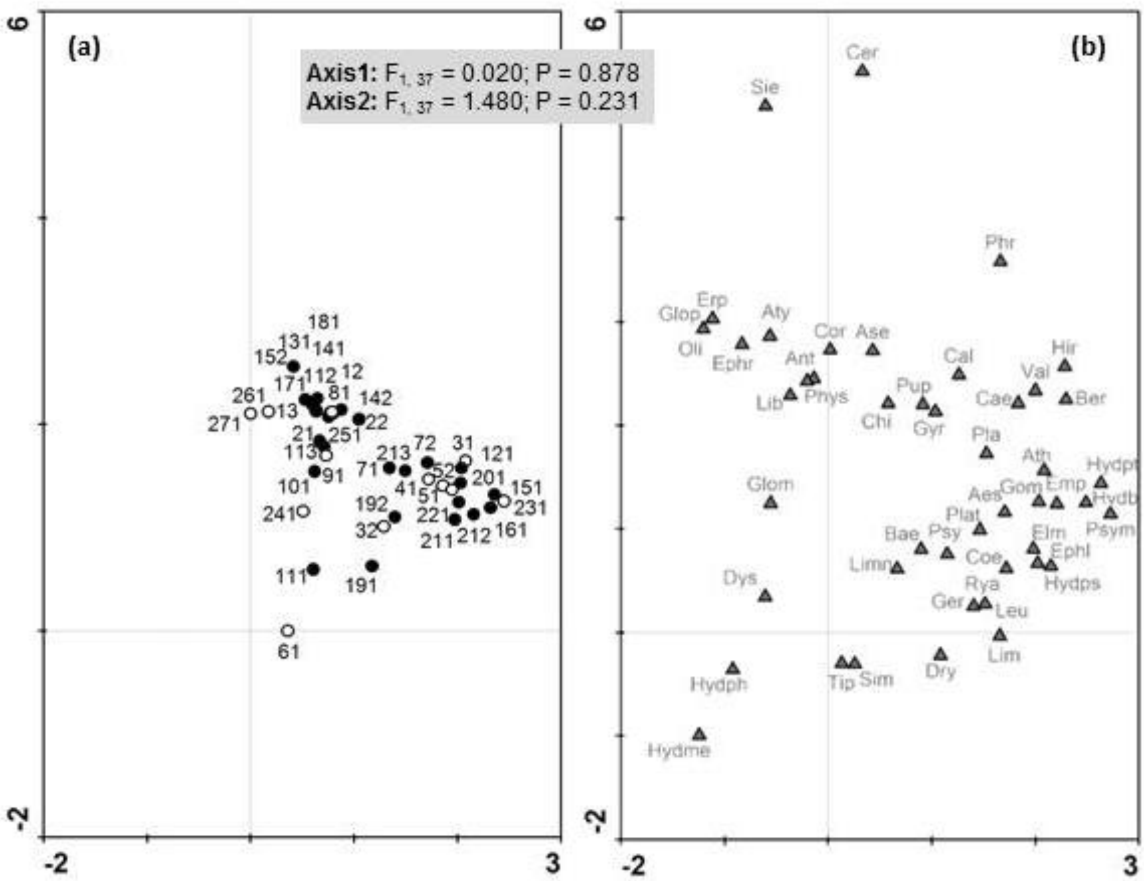


Fig. 5. DCA scatterplots representing the distribution of sampling sites (a) according to the abundance of macroinvertebrate families (b). The plots were post-edited to distinguish sites invaded (black circles/bars) and non-invaded (white circles/bars) by the Asian clam. As in previous figures, the dots were excluded from the site's ID for clarity purposes in (a) and macroinvertebrate abbreviations in (b) can be found decoded in Table S6. Site 1.1 was excluded from the analysis because there was no other group than Corbiculidae. The one-way ANOVA summary distinguishing invaded and non-invaded sites can be found in the top of the plots shadowed grey.

Table 2. Summary of Canonical Correspondence Analysis (CCA) applied to the benthic invertebrate dataset, showing partitioning of variation between environmental and clam presence/abundance data. For each explanatory dataset, full and partial models are shown, as well as their significance (Monte Carlo permutation test, $P \leq 0.05$). Partial models represent the variation due to one subset after excluding the shared component of variation with the other subset.

Source of variation	Eigenvalue	Total inertia (total variation)	*F*	*P*	Proportion of variance explained
Environment	1.675	2.307	5.8	0.002	72.6%
Environment | Clam	1.510	2.307	5.3	0.002	65.5%
Clam	0.231	2.307	2.0	0.062	10.0%
Clam | Environment	0.066	2.307	1.4	0.100	2.9%

Contradicting the lack of relationship between clam data and the macroinvertebrate community structure, clam density in invaded sites was significantly correlated with axis 1 scores from DCA (Table 1). This mostly corresponds to a moderate association ($r = 0.463$; see Figure S1), which seems to translate into an increasing trend in *C. fluminea* density from left to the right side of the sample scatterplot, e.g. site 12.1 with more than 1000 individuals m^{-2}, and then 2.2, 14.2, 15.1, 16.1, 19.1 and 21.2 with clam densities between 100 and 1000 individuals m^{-2} (Figure 5a). Thus, higher *C. fluminea* densities were associated with scraper grazers (e.g. Hydroptilidae, Limnephilidae) but, mostly, collector gatherers (e.g. Beraeidae, Elmidae, Ephemerellidae, Hydrobiidae, Psychomyiidae); these taxa are on the right side of the DCA biplot. On the other hand, predators (e.g. Atyidae, Ceratopogonidae, Erpobdellidae, Glossiphoniidae) and shredders (e.g. Antomyiidae, Asellidae) were commoner on the opposite side of the biplot (Figure 5b), generally corresponding to sites with lower clam density. No bivalves other than *C. fluminea* were found; other molluscs, namely gastropods, were generally rare throughout the sampled catchment except for Hydrobiidae, which was abundant and widespread.

Discussion

The spatial distribution pattern of Asian clams in the studied channel network shows population connectedness, without downstream discontinuity; when absent, they are so in upstream tributaries within heavily invaded sub-catchments (9.1 and 23.1) or in entire system (RR and VV) of the catchment main channels. This primarily confirms passive downstream dispersal with water currents as the main mechanism for local dispersal of *C. fluminea*; such a dispersal pathway is actually recognised of major relevance for the species even in lakes (Hoyer et al., 2015).

Spatial discontinuity in the overall clam density distribution within the drainage catchment seems hence independent from a putative barrier effect of dikes. Therefore, the environmental heterogeneity among sites, if confirmed, would be the likely player defining invading populations. In this way, the role of sediment as a major determinant of clam distribution and density was an expected figure, but this was not confirmed in the present study. Although the Asian clam seems to prefer finer sediments (Belanger, Farris, Cherry, & Cairns, 1985; Cooper 2007; Schmidlin & Baur, 2007; Lucy et al., 2012), the studied area is mostly homogeneous with coarse- or medium-granule sand substrates that often sustaining dense populations. A relationship between population distribution and sediment organic load was also expected since *C. fluminea* juveniles and adults are capable of both filter- and pedal-feeding (Reid, McMahon, Foighil, & Finnigan, 1992), the latter being relevant when planktonic resources are short (Hakenkamp & Palmer, 1999) as in the studied catchment. However, no association could be identified. Clear positive correlations between clam distribution (e.g. biomass, growth rates) and sediment organic load were obtained previously in systems with larger ranges and higher upper records of the latter (Sousa, Rufino, Gaspar, Antunes, & Guilhermino, 2008e). There are also examples where sediment organic matter ranges were similar to those obtained here and could constrain clam’s distribution (e.g., Sousa, Antunes, & Guilhermino, 2006), but others showing no significant correlation (e.g., Boltovskoy, Correa, Cataldo, Stripeikis, & tudino, 1997).

Unlike sediment, water physico-chemistry and channel hydromorphology bear some influence in clam density distribution. A noticeable exception to this pattern is the clear separation of the non-invaded RR sites by the water physico-chemistry (3.1–6.1; see Figure 3b). However, a comparative analysis of the records regarding these variables (Table S2) and *C. fluminea* tolerance ranges evidences the frailty of such a separation. Considering the requirements of clam shell production, water mineralisation has been recognised meaningful in constraining the establishment of the invader. For example, Sousa et al. (2008e) positively correlated hardness with *C. fluminea* density and their records were consistently lower than those obtained here for the non-invaded system; and indeed, the ranges found in our sites for hardness and calcium content are above lower limits defining ideal conditions for the establishment of the invader (Ilarri & Sousa, 2012). As well, pH is far from the assumed lower limit of tolerance of 5.6 (Karatayev, Burlakova, & Padilla, 2005) and dissolved oxygen levels found in these sites were always above 7 mg L^{-1}, which is clearly above both the levels limiting the species growth ranging within 1-3 mg L^{-1} (Belanger, 1991) and the levels configuring high potential for massive invasion (Ilarri & Sousa, 2012). As far as we could reach in the literature, upper limits of tolerance to dissolved organic carbon or turbidity for the Asian clam have not been established yet but are assumed high (Way, Hornbach, Millerway, Payne, & Miller, 1990; Ilarri & Sousa, 2012) and certainly representing higher levels than those felt in non-invaded RR sites considering TSS and turbidity records. The relationship between nutrient load (P and N) and *C. fluminea* distribution was as well not clear in the present study but literature indicates that the relationship is still poorly understood. While Nakamura & Kerciku (2000) found minimum Chl*a* levels along with maximum nutrient (NH-N and PO-P) levels in sites within a lake where dense *Corbicula* patches stand, Pigneur et al. (2014) confirmed the increase of P levels by *C. fluminea*, linking it to reduced uptake of the nutrient by a depressed phytoplankton mass following consumption by clams.

Clam distribution responded mildly to channel hydromorphology; the single identified preference was towards deeper and wider channels (which are the main channels) with depth evidencing an incipient gradient (range: 0.10-0.80 m). In fact, *C. fluminea* populations have been found throughout an extended depth range as illustrated e.g. in the study by Minchin (2014) where clams established in shallow to 24-m depth waters. The apparent preference for wider channels can relate to an avoidance of shaded areas necessarily linked to lower water temperatures (*C. fluminea* prefers warmer waters in general; e.g. Simard et al. 2012), this being directly interpreted from an integrative reading of table S4. The gradient found for flow was wider and clams established in sites with practically absent flow as well as under truly lotic conditions (see Table S4), but this gradient did not correspond to any clear preference pattern. Also Boltovskoy et al. (1997) and Gangloff et al. (2011) couldn't correlate flow velocity with *C. fluminea* distribution in invaded systems. Although in the present study neither macrophytes nor filamentous algae seem to deter the Asian clam, the opposite trend is apparent from the literature (e.g., Karatayev, Burlakova, Kesterson, & Padilla, 2003). It is also reasonable to hypothesise that seasonal exudates from some macrophytes also have the potential to promote such segregation on the basis of allelopathy mechanisms (Stamp, 2003). Our sampling period may not have been coincident with the most favourable season establishing macrophyte-driven effects on the Asian clam distribution.

As to our knowledge this is the first study attempting to relate the Asian clam invasion with integrative biotic metrics *sensu* WFD either considering the macroinvertebrate or other benthic communities. Overall, the biological water quality seems largely unaffected by the invader in the present study. The clam's establishment in the system may eventually be recent (no historic information could be retrieved in the literature or with locals) and still facing the typical *lag* phase that constrains the overall growth of the population (Rosa et al., 2011), keeping low abundances with linked low ecological impacts. Site 12.1 stands against the odds by holding a highly dense, stable population (see an earlier study by Rosa et al., 2014). Here water quality was found moderate but there are not much sites with better classification and only one of these is a non-invaded site. These inconsistent evidences prevent a clear contribution to assess whether integrative water quality metrics are sensitive to the threat represented by a significant aquatic nuisance such as the Asian clam. Despite inducing major ecological damage (e.g., Sousa et al., 2014), the clam is also a powerful biofilter (see the comparison with zooplankton and/or unionids shorter top-down control abilities in e.g., Cohen, Dresler, Phillips, & Cory, 1984; Lopez et al., 2006) and water clearance is a major driver of ecological status improvement as understood by the regulatory framework. Moreover, the WFD approach to the monitoring of biotic communities is limited to the use of biotic indices, mostly developed to assess organic pollution (Chapman & Jackson, 1996). The use of these indices (e.g., EPT *taxa*) may produce confounding results, and may either over- or understate environmental quality, especially under multiple stress scenarios. Different stressors interact with each other, producing an unpredictable result in terms of the ecological-quality outcome (Vidal et al., 2014). A relevant add-on that may clarify on this problem could be the use of fine-resolution community-analysis tools, as employed in the present study (see below). This may improve the discriminatory power of bioassessments and prevent erroneous conclusions on the ecological health of the assessed ecosystem, in particular when stressors other than those centring the WFD methodological approach are into place (Vidal et al., 2014).

In contrast to the WFD approach, community-structure analysis enables the extraction of gradients that explain much better the proximity or separation among groups of samples, and their relationship with environmental parameters. In the present study, this approach showed more clearly that environmental variables rather than the clam presence or density were the major descriptors explaining the variation of the macroinvertebrate communities within the studied catchment (note that only a low percentage of the total variation in the CCA analysis remained unexplained). Still, a mild association was found between the invader and macroinvertebrate species belonging to specific functional feeding groups, namely scrapper grazers and collector gatherers.

The benefit of collector gatherers and consequently predators has been identified as a general asset of freshwater bivalves given their role as benthic-pelagic couplers (e.g. Howard & Cuffey, 2006). In fact, different studies have shown so far that Asian clam density in invaded ecosystems has been indeed positively related to macroinvertebrate communities in general or the abundance of particular groups (Soucek, Schmidt, & Cherry, 2001; Howard & Cuffey, 2006; Ilarri et al., 2012; Schmidlin, Schmera, & Baur, 2012; Sampaio & Rodil, 2014), this being linked to the ability of the clam as an ecosystem engineer. On the one hand, *C. fluminea* is a major actor in the organic matter dynamics within the benthic-pelagic interface by significantly contributing to the enrichment of the benthic compartment with organic matter from clam faeces and pseudofaeces (Sousa et al., 2009), which can favour other macroinvertebrates. Evidences generated so far are not emphatic in supporting this enrichment service (see e.g., Werner & Rothhaupt, 2007) mainly because of the counterweight represented by clam intensive pedal feeding on benthic bacteria, periphyton and own deposited matter (Hakenkamp & Palmer, 1999; Hakenkamp et al., 2001). On the other hand, shell deposition by dense populations that periodically undergo massive die-off events drive structural changes in the substrate, particularly evident in sandy systems, implying e.g. increased provision of substrata for attachment and refuge (Gutierrez, Jones, Strayer, Iribarne, 2003; Sousa et al., 2009; Schmidlin et al., 2012; Bódis, Tóth, Szekeres, Borza, & Sousa, 2014).

Amphipods and chironomidae dipterans seem to be favoured by both clam-driven organic matter enhancement and sediment restructuring (Werner & Rothhaupt, 2008; Ilarri et al., 2012, 2014; Sampaio & Rodil, 2014), but the positive association with the former is largely dependent on functional specificity of the species involved (Sampaio & Rodil, 2014). Negative effects by *C. fluminea* in macroinvertebrate groups with sand preference were also identified (Schmidlin et al., 2012), and density of the ephemeropteran *Caenis* sp. that prefers hard substrates increased with post die-off shell loading of sandy substrates (Werner & Rothhaupt, 2007), which confirms the relevance of the invaders role as a habitat restructurer in influencing benthic macroinvertebrate communities. Gastropods, composing the other molluscs found in this study (with Bivalvia represented only by *C. fluminea*), have been positively associated with the clam invader on the basis of shell use for refuge, scrapper grazing and oviposition, plus as recipients of clam-driven biodeposition benefits (Ilarri et al., 2012; Sampaio & Rodil, 2014). Unfortunately, we did not quantify the empty-shell cover in the present study. Still, it is reasonable to highlight 12.1 as a site where the effect of shells can be relevant given the immediately identifiable amounts involved; these are by far larger than in any other sampled site. Therefore, the mild trend for higher *C. fluminea* densities to favour scrappers and gatherers should mostly relate to enhanced biodeposition and bioturbation of sediment operated by the clam.

Although a part of the benthic biota can be favoured by the clam invader, detrimental effects related to its presence should not be disregarded. This has been primarily evidenced by the strong relationship between *C. fluminea* establishment and decline of native, often protected bivalves (Sousa, Antunes, & Guilhermino, 2007; Sousa et al., 2008a; c, 2011; Ilarri et al., 2012), which contribute to the typical molluscan diversity of undisturbed, highly valuable wetland systems and drainage catchments (Prezant & Chapman, 2004; Herzon & Helenius, 2008; Sousa, dias, guilhermino, & Antunes, 2008b). Such a molluscan diversity was clearly not observed in the present study. No bivalves other than *C. fluminea* were found; other molluscs, namely gastropods, were generally rare except for Hydrobiidae, which was abundant and widespread. This can be linked to unmonitored loading of the system from diffuse pollution sources (e.g., agricultural fields in the surroundings) affecting species with lower tolerance to organic contamination than those found here - Physidae, Planorbidae, Hidrobiidae and Valvatidae gastropods are tolerant *taxa* (SNIFFER, 2007). Also, sequent dikes constrain fish migration within the system, which may prevent the establishment of mussel species reproducing via glochidia larvae requiring a suitable fish host to complete the life cycle as discussed by Gangloff et al. (2011). Finally, it is worth remarking the strong effect that the invasive clam may have in benthic bacteria and periphyton communities through its pedal-feeding activity, particularly in systems where water column resources are scarce (Hakenkamp et al., 2001), the latter being a keystone component of biological quality assessment *sensu* WFD. Therefore, the nuisance potential of the freshwater invader must not be disregarded but rather studied in the future with enhanced focus on specific biota such as periphytic communities. Such future studies may provide extended cues on the sensitivity of WFD assessment methodologies to a significant stressor in our freshwaters nowadays, i.e. biological invasions.

Acknowledgements

This work was supported by European Funds through COMPETE and by National Funds through the Portuguese Science Foundation (FCT) within project PTDC/AAC-AMB/113515/2009 (project CONTROLCLAM).

References

Alba-Tercedor, J., & Sánchez-Ortega, A. (1988). A simple and quick method to evaluate biological quality of running freshwater based on Hellawell (1978). *Limnética*, 4, 51–56.

Aldridge, D.W., & McMahon, R.F. (1978). Growth, fecundity, and bioenergetics in a natural population of the Asiatic freshwater clam, *Corbicula manilensis* Philippi, from north central Texas. *Journal of Mollus*, 44, 49–70.

APHA (1995). *Standard Methods for the Examination of Water and Wastewater*. Washington: American Public Health Association.

Araujo, R., Moreno, D., & Ramos, M.A. (1993). The Asiatic clam *Corbicula fluminea* (Müller, 1774) (Bivalvia: Corbiculidae) in Europe. *American Malacological Bulletin*, 10, 39–49.

Barbour, M.T., Gerritsen, J., Snyder, B.D. & Stribling, J.B. (1999). *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish*. Washington: Environmental Protection Agency; Office of Water.

Belanger, S.E. (1991). The effect of dissolved-oxygen, sediment, and sewage-treatment plant discharges upon growth, survival and density of Asiatic clams. *Hydrobiologia*, 218, 113–126.

Belanger, S.E., Farris, J.L., Cherry, D.S., & Cairns, J. (1985). Sediment preferences of the freshwater Asiatic clam, *Corbicula fluminea*. *Nautilus*, 99, 66–73.

Belz, C.E., Darrigran, G., Netto, O.S.M., Boeger, W.A. & Ribeiro, P.J. (2012). Analysis of Four Dispersion Vectors in Inland Waters: The Case of the

Invading Bivalves in South America. *Journal of Shellfish Research*, 31, 777–784.

Bodamer, B.L., & Bossenbroek, J.M. (2008). Wetlands as barriers: Effects of vegetated waterways on downstream dispersal of zebra mussels. *Freshwater Biology*, 53, 2051–2060.

Bódis, E., Tóth, B., Szekeres, J., Borza, P., & Sousa, R. (2014). Empty native and invasive bivalve shells as benthic habitat modifiers in a large river. *Limnologica - Ecology and Management of Inland Waters*, 49, 1–9.

Boltovskoy, D., Correa, N., Cataldo, D., Stripeikis, J., & Tudino, M. (1997). Environmental stress on *Corbicula fluminea* (Bivalvia) in the Paran River delta (Argentina): complex pollution-related disruption of population structures. *Arch. Hvdrobiol.*, 138, 483–507.

Borcard, D., Legendre, P., & Drapeau, P. (1992) Partialling out the spatial component of ecological variation. *Ecology*, 73, 1045–1055.

Brancotte, V., & Vincent, T. (2002). The invasion of french hydrographic system by the molluscs Corbicula spp details of settlement and main role of ship canals. *Bulletin Francais De La Peche Et De La Pisciculture*, 325–337.

Britton, J.C. (1982). Biogeography and Ecology of the Asiatic clam, Corbicula, in Texas. In J.R. Davis (Ed.) *Proceedings of the Symposium on Recent Benthological Investigations in Texas and adjacent States*, Texas Academy of Science, Austin, 21–31.

Britton, J.C., & Morton, B. (1982). A dissection guide, field and laboratory manual for the introduced bivalve *Corbicula fluminea*. *Malacological Review Supplement*, 1–82.

Brower, J.E., Zar, J.H., & von Ende, C. (1998). *Field and Laboratory Methods for General Ecology*. Boston: WCB McGraw-Hill.

Cantanhêde, G., Hahn, N.S., Gubiani, É.A., & Fugi, R. (2008). Invasive molluscs in the diet of *Pterodoras granulosus* (Valenciennes, 1821) (Pisces, Doradidae) in the Upper Paraná River floodplain, Brazil. *Ecology of Freshwater Fish*, 17, 47–53.

Castro, B.B., & Gonçalves, F. (2011) Planktivory in non-indigenous fish and implications for trophic interactions in a Mediterranean shallow lake. *Annales de Limnologie - International Journal of Limnology*, 47, 269–280.

Chapman, D.V., & Jackson, J. (1996). Biological monitoring. In J. Bartram & R. Ballance (Eds.), *Water quality monitoring. A practical guide to the design and implementation of freshwater quality studies and monitoring programmes* (pp. 263–302). London, UK: E & FN Spon.

Cherry, D.S., Scheller, J.L., Cooper, N.L., & Bidwell, J.R. (2005). Potential effects of Asian clam (*Corbicula fluminea*) die-offs on native freshwater mussels (Unionidae) I: water-column ammonia levels and ammonia toxicity. *Journal of the North American Benthological Society*, 24, 369–380.

Cohen, R.R.H., Dresler, P. V, Phillips, E.J.P., & Cory, R.L. (1984). The effect of the Asiatic clam, *Corbicula fluminea*, on the phytoplankton of the Potomac river, Maryland. *Limnology and Oceanography*, 29, 170–180.

Cooper, J.E. (2007). *Corbicula fluminea* (Asian clam) in the Roanoke River, North Carolina: A stressed population? *Southeastern Naturalist*, 6, 413–434.

Cooper, N.L., Bidwell, J.R., & Cherry, D.S. (2005). Potential effects of Asian clam (*Corbicula fluminea*) die-offs on native freshwater mussels (Unionidae) II: porewater ammonia. *Journal of the North American Benthological Society*, 24, 381–394.

DAISIE (2008). *Corbicula fluminea*. Retrieved from http://www.europe-aliens.org.

Darrigran, G. (2002). Potential impact of filter-feeding invaders on temperate inland freshwater environments. *Biological Invasions*, 4, 145–156.

Davis, M.A. (2009). *Invasion Biology*. Oxford: Oxford University Press.

Ebina, J., Tsutsui, T., & Shirai, T. (1983). Simultaneous determination of total nitrogen and total phosphorus in water using peroxodisulfate oxidation. *Water Research*, 17, 1721–1726.

Edington, J.M., & Hildrew, A.G. (2005) *A Revised Key to the Caseless Caddis Larvae of the British Isles*. Cumbria: Freshwater Biological Association.

Elliott, J.M., & Humpesch, U.H. (2010), *Mayfly Larvae (Ephemeroptera) of Britain and Ireland: Keys and a Review of Their Ecology* (ed N 66 Scientific

Publications of the Freshwater Biological Association). Cumbria: Freshwater Biological Association.

Gabriel, K.R. (2002). Goodness of fit of biplots and correspondence analysis. *Biometrika*, 89, 423–436.

Gangloff, M.M., Hartfield, E.E., Werneke, D.C., & Feminella, J.W. (2011). Associations between small dams and mollusk assemblages in Alabama streams. *Journal of the North American Benthological Society*, 30, 1107–1116.

Gatlin, M.R., Shoup, D.E., & Long, J.M. (2013). Invasive zebra mussels (*Driessena polymorpha*) and Asian clams (*Corbicula fluminea*) survive gut passage of migratory fish species: Implications for dispersal. *Biological Invasions*, 15, 1195–1200.

Grayson, R.B., Gippel, C.J., Finlayson, B.L., & Hart, B.T. (1997). Catchment-wide impacts on water quality: the use of “snapshot” sampling during stable flow. *Journal of Hydrology*, 199,121–134.

Gutierrez, J.L., Jones, C.G., Strayer, D.L. & Iribarne, O.O. (2003) Mollusks as ecosystem engineers: the role of shell production in aquatic habitats. *Oikos*, 101, 79–90.

Hakenkamp, C.C., & Palmer, M.A. (1999). Introduced bivalves in freshwater ecosystems: The impact of Corbicula on organic matter dynamics in a sandy stream. *Oecologia*, 119, 445–451.

Hakenkamp, C.C., Ribblett, S.G., Palmer, M. A., Swan, C.M., Reid, J.W., & Goodison, M.R. (2001). The impact of an introduced bivalve (*Corbicula fluminea*) on the benthos of a sandy stream. *Freshwater Biology*, 46, 491–501.

Hering, D., Buffagni, A., Moog, O., Sandin, L., Sommerhäuser, M., Stubauer, I., … Zahrádková, S. (2003). The Development of a System to Assess the Ecological Quality of Streams Based on Macroinvertebrates – Design of the Sampling Programme within the AQEM Project. *Internat. Rev. Hydrobiol.*, 88, 345–361.

Herzon, I., & Helenius, J. (2008). Agricultural drainage ditches, their biological importance and functioning. *Biological Conservation*, 141, 1171–1183.

Howard, J.K., & Cuffey, K.M. (2006). The functional role of native freshwater mussels in the fluvial benthic environment. *Freshwater Biology*, 51, 460–474.

Hoyer, A.B., Schladow, S.G., & Rueda, F.J. (2015). Local dispersion of nonmotile invasive bivalve species by wind-driven lake currents. *Limnology and Oceanography*, n/a–n/a.

Hynes, H.B.N. (1993). *A Key to the Adults and Nymphs of the British Stoneflies (Plecoptera): With Notes on Their Ecology and Distribution*. Cumbria: Freshwater Biological Association.

Ilarri, M.I., Freitas, F., Costa-Dias, S., Antunes, C., Guilhermino, L., & Sousa, R. (2012). Associated macrozoobenthos with the invasive Asian clam *Corbicula fluminea*. *Journal of Sea Research*, 72, 113–120.

Ilarri, M., & Sousa, R. (2012). *Corbicula fluminea* Muller (Asian clam). *A Handbook of Global Freshwater Invasive Species*. London: Earthscan.

Ilarri, M.I., Souza, A. T., Antunes, C., Guilhermino, L., & Sousa, R. (2014). Influence of the invasive Asian clam *Corbicula fluminea* (Bivalvia: Corbiculidae) on estuarine epibenthic assemblages. *Estuarine, Coastal and Shelf Science*, 143, 12–19.

INAG, I.P. (2008a). *Protocolo de amostragem e análise para os macroinvertebrados bentónicos*. Lisboa: Ministério do Ambiente, Ordenamento do Território e do Desenvolvimento Regional.

INAG, I.P. (2008b) *Tipologia de Rios Em Portugal Continental No âmbito Da Implementação Da Diretiva Quadro Da Água. I - Caracterização Abiótica*. Lisboa: Ministério do Ambiente, Ordenamento do Território e do Desenvolvimento Regional.

INAG, I.P. (2009). *Critérios Para a Classificação Do Estado Das Massas de água Superficiais*. Lisboa: Ministério do Ambiente, Ordenamento do Território e do Desenvolvimento Regional.

Ingram, W.M. (1959). Asiatic clams as potential pests in California Water supplies. *Journal of the American Water Works Association*, 51, 363–370.

Jaímez-Cuéllar, P., Vivas, S., Bonada, N., Robles, S., Mellado, A., AÍvarez, M., … Alba-Tercedor, J. (2002). Protocolo GUADALMED (PRECE). *Limnetica*, 21, 187–204.

Jenner, H.A., Whitehouse, J.W., Taylor, C.J.L., & Khalanski, M. (1998). Cooling water management in European power stations Biology and control of fouling. *Hydroécologie Appliquée*, 10, 1–225.

JRC (2009) Water Framework Directive intercalibration technical report - Part 1: Rivers. Luxembourg: Official Publications of the European Communities.

Kappes, H., & Haase, P. (2012) Slow, but steady: dispersal of freshwater molluscs. *Aquatic Sciences*, 74, 1–14.

Karatayev, A.Y., Burlakova, L.E., Kesterson, T., & Padilla, D.K. (2003) Dominance of the Asiatic clam, *Corbicula fluminea* (Muller), in the benthic community of a reservoir. *Journal of Shellfish Research*, 22, 487–493.

Karatayev, A.Y., Burlakova, L.E., & Padilla, D.K. (2005). Contrasting distribution and impacts of two freshwater exotic suspension feeders, Dreissena polymorpha and *Corbicula fluminea*. *The comparative roles of suspension-feeders in ecosystems - Proceedings of the NATO Advanced Research Workshop on The Comparative Roles of Suspension-Feeders in Ecosystems, Nida, Lithuania, 4-9 October 2003* (eds R.F. Dame), & S. Olenin), pp. 239–262. Springer Netherlands.

Karatayev, A.Y., Padilla, D.K., Minchin, D., Boltovskoy, D., & Burlakova, L.E. (2007). Changes in global economies and trade: the potential spread of exotic freshwater bivalves. *Biological Invasions*, 9, 161–180.

Kraemer, L.R., & Galloway, M.L. (1986). Larval development of *Corbicula fluminea* (Muller) (Bivalvia, Corbiculacea) - An appraisal of its heterochrony. *American Malacological Bulletin*, 4, 61–79.

Kristensen, E., & Andersen, F.O. (1987). Determination of organic carbon in marine sediments: a comparison of two CHN-analyzer methods. *Journal of Experimental Marine Biology and Ecology*, 109, 15–23.

Leung, B., Lodge, D.M., Finnoff, D., Shogren, J.F., Lewis, M.A., & Lamberti, G. (2002). An ounce of prevention or a pound of cure: bioeconomic risk analysis of invasive species. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 269, 2407–2413.

Lind, O.T. (1979). *Handbook of Common Methods in Limnology*. St. Louis: Mosby.

Lopez, C.B., Cloern, J.E., Schraga, T.S., Little, A.J., Lucas, L. V, Thompson, J.K., & Burau, J.R. (2006). Ecological values of shallow-water habitats: Implications for the restoration of disturbed ecosystems. *Ecosystems*, 9, 422–440.

Lorenzen, C.J. (1967). Determination of Chlorophyll and Pheo-Pigments: Spectrophotometric Equations. *Limnology and Oceanography*, 12, 343–346.

Lucy, F.E., Karatayev, A.Y., & Burlakova, L.E. (2012). Predictions for the spread, population density, and impacts of *Corbicula fluminea* in Ireland. *Aquatic Invasions*, 7, 465–474.

Mackie, G.L., & Claudi, R. (2010). *Monitoring and Control of Macrofouling Mollusks in Fresh Water Systems*. London: CRC Press Inc.

Marsh, P.C. (1985). Secondary production of introduced Asiatic clam, *Corbicula fluminea*, in a central Arizona canal. *Hydrobiologia*, 124, 103–110.

McMahon, R.F. (2002). Evolutionary and physiological adaptations of aquatic invasive animals: selection versus resistance. *Canadian Journal of Fisheries and Aquatic Sciences*, 59, 1235–1244.

Minchin, D. (2014). The distribution of the Asian clam *Corbicula fluminea* and its potential to spread in Ireland. *Management of Biological Invasions*, 5, 165–177.

Mouthon, J. (2003). Longitudinal and temporal variations of density and size structure of *Corbicula fluminea* (Bivalvia) populations in the Saône and Rhône rivers (France). *Annales de Limnologie - International Journal of Limnology*, 39, 15–25.

Munné, A. & Prat, N. (2009) Use of macroinvertebrate-based multimetric indices for water quality evaluation in Spanish Mediterranean rivers: An intercalibration approach with the IBMWP index. *Hydrobiologia*, 628, 203–225.

Nakamura, Y., & Kerciku, F. (2000). Effects of filter-feeding bivalves on the distribution of water quality and nutrient cycling in a eutrophic coastal lagoon. *Journal of Marine Systems*, 26, 209–221.

Pawley, S., Dobson, M., & Fletcher, M. (2011). *Guide to the British Freshwater Macroinvertebrates for Biotic Assessment*. Cumbria: Freshwater Biological Association.

Phelps, H.L. (1994). The Asiatic clam (*Corbicula fluminea*) invasion and system level ecological in the Potomac river estuary near Washington, DC. *Estuaries*, 17, 614–621.

Pigneur, L.-M., Falisse, E., Roland, K., Everbecq, E., Deliège, J.-F., Smitz, J.S., … Descy, J.P. (2014). Impact of invasive Asian clams, Corbicula spp., on a large river ecosystem. *Freshwater Biology*, 59, 573–583.

Pimentel, D., Zuniga, R., & Morrison, D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics*, 52, 273–288.

Pope, L.J., Eliason, A.H., Fredericks, J.J., Rendings, R.R., Blackwood, D., & Poloni, C.F. (2000). Grain-size analysis of marine sediments - methodology and data processing. *USGS East-coast sediment analysis: procedures, database, and georeferenced displays*, U.S. Geolo U.S. Geological Survey.

Prezant, R.S., & Chalermwat, K. (1984). Flotation of the bivalve *Corbicula fluminea* as a means of dispersal. *Science*, 225, 1491–1493.

Prezant, R.S., & Chapman, E.J. (2004). Freshwater molluscs of the United States Military Academy drainages (West point, NY) and comparative regional biodiversity of gastropods. *Northeastern Naturalist*, 11, 273–294.

Prokopovich, N.P., & Hebert, D.J. (1965). Sedimentation in the Delta–Mendota Canal. *Journal of the American Water Works Association*, 57, 375–382.

Raven, P.J., Dawson, F.H., & Everard, M. (1998) Quality assessment using River Habitat Survey data. *Aquatic Conservation – Marine and Freshwater Ecosystems*, 8, 477–499.

Reid, R.G.B., McMahon, R.F., Foighil, D.O., & Finnigan, R. (1992). Anterior inhalant currents and pedal feeding in bivalves. *Veliger*, 35, 93–104.

Rosa, I.C., Pereira, J.L., Gomes, J., Saraiva, P.M., Gonçalves, F., & Costa, R. (2011). The Asian clam *Corbicula fluminea* in the European freshwater-dependent industry: A latent threat or a friendly enemy? *Ecological Economics*, 70, 1805–1813.

Rosa, I.C., Pereira, J.L., Costa, R., Gomes, J., de Lourdes Pereira, M., & Gonçalves, F. (2014). Dispersal of *Corbicula fluminea* : factors influencing the invasive clam's drifting behavior. *Annales de Limnologie - International Journal of Limnology*, 50, 37–47.

Sampaio, E., & Rodil, I.F. (2014). Effects of the invasive clam *Corbicula fluminea* (Müller, 1774) on a representative macrobenthic community from two estuaries at different stages of invasion. *Limnetica*, 33, 249–262.

Schmidlin, S., & Baur, B. (2007). Distribution and substrate preference of the invasive clam *Corbicula fluminea* in the river Rhine in the region of Basel (Switzerland, Germany, France). *Aquatic Sciences*, 69, 153–161.

Schmidlin, S., Schmera, D., & Baur, B. (2012). Alien molluscs affect the composition and diversity of native macroinvertebrates in a sandy flat of Lake Neuchâtel, Switzerland. *Hydrobiologia*, 679, 233–249.

Serra, S., Coimbra, N., & Graça, M. (2009). *Invertebrados de Água Doce: Chave de Identificação Das Principais Famílias*. Coimbra: Imprensa da Universidade de Coimbra.

Simard, A., Paquet, A., Jutras, C., Robitaille, Y., Blier, P., Courtois, R. & Martel, A. (2012). North American range extension of the invasive Asian clam in a St. Lawrence River power station thermal plume. *Aquatic Invasions*, 7, 81–89.

SNIFFER (2007). *Revision and Testing of BMWP Scores - Final Report, Project WFD72A*. Edinburgh.

Soucek, D.J., Schmidt, T.S., & Cherry, D.S. (2001). In situ studies with Asian clams (*Corbicula fluminea*) detect acid mine drainage and nutrient inputs in low-order streams. *Canadian Journal of Fisheries and Aquatic Sciences*, 58, 602–608.

Sousa, R., Antunes, C., & Guilhermino, L. (2006). Factors influencing the occurrence and distribution of *Corbicula fluminea* (Muller, 1774) in the River

Lima estuary. *Annales De Limnologie-International Journal of Limnology*, 42, 165–171.

Sousa, R., Antunes, C., & Guilhermino, L. (2007). Species composition and monthly variation of the Molluscan fauna in the freshwater subtidal area of the River Minho estuary. *Estuarine, Coastal and Shelf Science*, 75, 90–100.

Sousa, R., Antunes, C., & Guilhermino, L. (2008). Ecology of the invasive Asian clam *Corbicula fluminea* (Muller, 1774) in aquatic ecosystems: an overview. *Annales De Limnologie-International Journal of Limnology*, 44, 85–94.

Sousa, R., Dias, S., Freitas, V., & Antunes, C. (2008a). Subtidal macrozoobenthic assemblages along the River Minho estuarine gradient (north-west Iberian Peninsula). *Aquatic Conservation-Marine and Freshwater Ecosystems*, 18, 1063–1077.

Sousa, R., Dias, S.C., Guilhermino, L., & Antunes, C. (2008b). Minho River tidal freshwater wetlands: threats to faunal biodiversity. *Aquatic Biology*, 3, 237–250.

Sousa, R., Morais, P., Antunes, C., & Guilhermino, L. (2008c). Factors Affecting *Pisidium amnicum* (Muller, 1774; Bivalvia: Sphaeriidae) Distribution in the River Minho Estuary: Consequences for its Conservation. *Estuaries and Coasts*, 31, 1198–1207.

Sousa, R., Nogueira, A.J.A., Gaspar, M.B., Antunes, C., & Guilhermino, L. (2008d). Growth and extremely high production of the non-indigenous invasive species *Corbicula fluminea* (Muller, 1774): Possible implications for ecosystem functioning. *Estuarine Coastal and Shelf Science*, 80, 289–295.

Sousa, R., Rufino, M., Gaspar, M., Antunes, C., & Guilhermino, L. (2008e). Abiotic impacts on spatial and temporal distribution of *Corbicula fluminea* (Muller, 1774) in the River Minho Estuary, Portugal. *Aquatic Conservation-Marine and Freshwater Ecosystems*, 18, 98–110.

Sousa, R., Gutiérrez, J.L., & Aldridge, D.C. (2009). Non-indigenous invasive bivalves as ecosystem engineers. *Biological Invasions*, 11, 2367–2385.

Sousa, R., Ilarri, M., Souza, A.T., Antunes, C., & Guilhermino, L. (2011). Rapid decline of the greater European peaclam at the periphery of its distribution. *Annales De Limnologie-International Journal of Limnology*, 47, 211–219.

Sousa, R., Novais, A., Costa, R., & Strayer, D.L. (2014). Invasive bivalves in fresh waters: Impacts from individuals to ecosystems and possible control strategies. *Hydrobiologia*, 735, 233–251.

Stamp, N. (2003). Out of the Quagmire of Plant Defense Hypotheses. *The Quarterly Review of Biology*, 78, 23–55.

Strayer, D.L. (1999). Effects of alien species on freshwater mollusks in North America. *Journal of the North American Benthological Society*, 18, 74–98.

Sundermann, A., Lohse, S., Beck, L. A., & Haase, P. (2007). Key to the larval stages of aquatic true flies (Diptera), based on the operational taxa list for running waters in Germany. *Annales de Limnologie - International Journal of Limnology*, 43, 61–74.

Tachet, H. (2000). *Invértebrés D'eau Douce: Systématique, Biologie, écologie*. CNRS Editions.

Ter Braak, C.J.F. (1988) Partial canonical correspondence analysis. In H. H. Brock (Ed.), *Classification and related methods of data analysis* (pp. 551–558). Amsterdam: North-Holland.

Ter Braak, C.J.F. (1995). Ordination. In: R.H.G. Jongman, C.J.F. Ter Braak, & O.F.R. Von Tongeren (Eds), *Data analysis in community and landscape ecology* (pp. 91–173). Cambridge: Cambridge University Press.

Vaughn, C.C., & Hakenkamp, C.C. (2001). The functional role of burrowing bivalves in freshwater ecosystems. *Freshwater Biology*, 46, 1431–1446.

Vidal, T., Santos, J.I., Marques, C.R., Pereira, J.L., Claro, M.T, Pereira, R., … Gonçalves, F. (2014). Resilience of the macroinvertebrate community of a small mountain river (Mau River, Portugal) subject to multiple stresses. *Marine and Freshwater Research*, 65, 1-12.

Voelz, N.J., McArthur, J.V., & Rader, R.B. (1998). Upstream mobility of the Asiatic clam *Corbicula fluminea*: Identifying potential dispersal agents. *Journal of Freshwater Ecology*, 13, 39–45.

Wallace, I.D., Wallace, B., & Philipson, G.N. (2003). *Keys to the Case-Bearing Caddis Larvae of Britain and Ireland*. Cumbria: Freshwater Biological Association.

Watson, A.M., & Ormerod, S.J. (2005). The distribution and conservation of threatened Sphaeriidae on British grazing marshland. *Biodiversity and Conservation*, 14, 2207–2220.

Way, C.M., Hornbach, D.J., Millerway, C.A., Payne, B.S., & Miller, A.C. (1990). Dynamics of Filter Feeding in *Corbicula fluminea* (Bivalvia, Corbiculidae). *Canadian Journal of Zoology-Revue Canadienne de Zoologie*, 68, 115–120.

Werner, S., & Rothhaupt, K.O. (2007). Effects of the invasive bivalve *Corbicula fluminea* on settling juveniles and other benthic taxa. *Journal of the North American Benthological Society*, 26, 673–680.

Werner, S., & Rothhaupt, K.O. (2008). Effects of the invasive Asian clam *Corbicula fluminea* on benthic macroinvertebrate taxa in laboratory experiments. *Fundamental and Applied Limnology*, 173, 145–152.

Williamson, C.E., Morris, D.P., Pace, M.L., & Olson, O.G. (1999). Dissolved organic carbon and nutrients as regulators of lake ecosystems: Resurrection of a more integrated paradigm. *Limnology and Oceanography*, 44, 795–803.

Suplementary Material

Table S1. Benthic macroinvertebrate *taxa* collected in a semi-natural drainage, in the littoral centre of Portugal.

Class/ Order	Family	1.1.	1.2.	1.3.	2.1.	2	3.1.	3	4.1.	5.1.	5.2.	6.1.	7.1.	7.2.	8.1.	9.1.	10.1.	11.1.	11.2.	11.3.	12.1.	13.1.	14.1.	14.2	15.1.	15.2.	16.1.	17.1.	18.1.	19.1.	19.2.	20.1.	21.1.	21.2.	21.3.	22.1.	23.1.	24.1.	25.1.	26.1.	27.1.
Bivalvia	Corbiculidae	3	15	36	15	24			1			1	11	51				3	7	1	9	6	6	2	1		1	1	6	6	2	5	5	4	6			24		1	
Coleoptera	Dryopidae											1																			1										
Coleoptera	Dytiscidae											1				4				1																					
Coleoptera	Elmidae					2	2	6	2	2						1		2				1	1							1	14	5	2	21	4	3	1				
Coleoptera	Gyrinidae								1											2											3									4	
Coleoptera	Hydrophilidae											2			1																										
Crustacea	Asellidae						2	2							2					7	33	1	1	12													1	5	2	168	
Crustacea	Atyidae														1					3								2	1												
Diptera	Athericidae										1																								1	1					
Diptera	Ceratopogonidae			1					1						1	12							1		4	34					2	1									
Diptera	Chironomidae		8	15	22	28	4	57	24	62	54	39	77	8	245	2092	5	25	29	134	91	248	42	92	153	274		42	297	5	67	29	3	39	41	4	25	26		317	165
Diptera	Diptera n.i.							2					2	1	8	36			3	8	1		2		7	5					4	2				1		1		6	4
Diptera	Empididae										1																														
Diptera	Limoniidae							1	2			7											1								3			6							
Diptera	Muscidae			1																																					
Diptera	Psychodidae				1																											1						1		1	
Diptera	Simuliidae			1	3			99	3	5	2	491	9			100	1	60	1	11	1			1	2					67	49		4	13	4	1	7	23		92	
Diptera	Tipulidae											3											1												1						
Ephemeroptera	Baetidae			1	1			11	4	22	37	70	24	1		996	2	14		28		3			10	15	3		1	71	71	5	7	126	3	3	19	5		57	135
Ephemeroptera	Caenidae					7	25	21			7		8		10	12		5	1	2	82	4	1	18	7	7			3	1	1									4	
Ephemeroptera	Ephemerellidae																													15	32		2	43	1	2	5	1			
Ephemeroptera	Ephemeridae																					4																			
Gastropoda	Hydrobiidae		1	2	2	4	50	116	48	172	221		66	16	17	112		3		2	459	2		7	2373	4	41		32	55	111	118	37	782	43	30	2846		1	45	2
Gastropoda	Physidae			2			2				1			1	21	12							2							2								1	4	16	12
Gastropoda	Planorbidae						1			1	1	2		1	9						4		4	1			1				4	3	1						4	1	2
Gastropoda	Valvatidae													1																											
Heteroptera	Corixidae														2									1																	
Heteroptera	Gerridae														1												2											4			
Heteroptera	Hydrometridae											2																													
Megaloptera	Sialidae														11											8															
Odonata	Aeshnidae																														1										
Odonata	Calopterygidae							1			2																								1	1				8	
Odonata	Coenagrionidae										1	1			2																1		2	1		1				1	
Odonata	Gomphidae																						1		1									1							
Odonata	Libellulidae														1	1																									
Odonata	Platycnemididae							2							1																										
Outros	Oligochaeta							3				2			2											38				1	4						3	2		230	187
Plecoptera	Leuctridae																													1											
Hirudinea	Erpobdellidae																																							8	
Hirudinea	Glossiphoniidae																																							17	
Hirudinea	Hirudinea n.i.						1																																		
Trichoptera	Beraeidae																				3																				
Trichoptera	Glossosomatidae																			1																					
Trichoptera	Hydropsychidae							1		1	7	1						2		6	1		2	4	1					2	12	1	2	98						1	
Trichoptera	Hydroptilidae																								1																
Trichoptera	Limnephilidae																																			1		2		2	
Trichoptera	Phryganeidae												1																												
Trichoptera	Psychomyiidae																																				2				
Trichoptera	Rhyacophilidae																													1											

Table S2. Water physico-chemistry of the sampled sites. Thirteen parameters were included in the water subset, namely temperature (Temp - ºC), conductivity (Cond - µScm^{-1}), pH, dissolved oxygen (Oxi - mg L^{-1}), alkalinity (Alkal - mg L^{-1}), calcium (Ca - mg L^{-1}), total phosphorous (TP - mg L^{-1}) and nitrogen content (TN - mg L^{-1}), hardness (Hard - mg L^{-1}), chlorophyll *a* (Chl*a* - µ gL^{-1}), total suspended solids (TSS – mg L^{-1}), coloured dissolved organic carbon (CDOC - m^{-1}) and turbidity (Turb - m^{-1}).

	Temp	Cond	pH	Oxi	Alkal	Ca	Hard	Chl*a*	TSS	TP	TN	CDOC	Turb
1.1	20.5	427	7.59	7.94	112	25	210	4.49	3.93	0.06	4.40	25.8	5.52
1.2	20.1	486	7.70	8.54	124	25	160	1.56	4.03	0.11	4.17	25.1	5.29
1.3	20.5	460	7.79	8.00	92	25	120	1.78	3.31	0.06	4.28	26.0	5.29
2.1	21.7	506	7.72	7.74	108	25	180	1.60	4.12	0.06	3.97	22.5	5.06
2.2	20.2	436	7.76	8.45	107	25	180	1.34	4.21	0.06	3.61	22.5	4.83
3.1	18.4	503	7.56	7.86	119	25	150	0.71	3.24	0.05	6.95	44.6	8.51
3.2	18	656	7.54	7.49	103	25	150	0.17	4.41	0.04	5.67	45.3	8.51
4.1	18.4	488	7.25	7.62	91	25	110	0.22	7.11	0.10	8.74	52.4	11.27
5.1	16.2	464	7.39	8.55	114	122	100	0.43	11.9	0.18	5.02	39.1	9.43
5.2	15.9	445	7.39	8.39	107	25	220	0.21	4.53	0.07	3.61	38.4	9.43
6.1	16.6	371	7.30	9.35	94	80	120	4.54	1.24	0.10	10.4	32.4	4.83
7.1	20.2	465	7.26	8.15	111	142	160	4.34	39.5	0.08	8.96	22.5	6.90
7.2	18.7	425	7.60	8.44	112	25	160	1.25	5.05	0.02	4.11	21.6	4.60
8.1	19.8	546	7.45	7.77	128	51	230	9.26	15.7	0.22	3.57	27.4	8.28
9.1	20.8	660	7.53	4.96	190	104	230	1.14	7.53	0.57	9.92	26.4	6.67
10.1	18.6	416	7.60	8.39	87	56	115	1.07	5.03	0.07	3.59	19.1	4.60
11.1	17.5	402	7.50	8.40	111	25	160	1.17	5.30	0.07	4.15	18.6	5.06
11.2	18.1	415	7.60	8.78	92.0	107	120	1.37	4.33	0.06	3.66	17.9	4.83
11.3	18.2	409	8.20	8.92	110	25	160	1.00	4.92	0.06	3.32	18.6	4.14
12.1	18.3	409	7.50	7.80	189	25	180	0.50	8.42	0.10	3.73	18.4	4.14
13.1	18.2	421	7.60	8.14	116	79	200	1.42	3.90	0.06	3.32	18.2	4.60
14.1	16.2	491	7.15	7.88	142	25	200	0.99	6.48	0.06	4.46	15.2	2.53
14.2	16.4	412	7.57	8.72	105	25	180	1.00	7.79	0.07	4.17	15.2	2.76
15.1	16.9	560	7.74	9.80	147	99	300	0.85	1.19	0.03	2.47	3.45	0.23
15.2	17.9	545	7.77	8.50	138	63	220	0.50	0.89	0.01	2.70	2.76	0.00
16.1	17.5	486	7.81	8.64	130	96	150	0.37	2.78	0.04	4.28	13.6	2.53
17.1	16.8	421	7.63	8.93	114	25	30	0.76	7.09	0.05	3.90	12.2	0.92
18.1	17.5	594	7.48	7.89	153	100	360	1.86	1.10	0.11	4.75	1.38	1.15
19.1	17.5	495	7.64	8.46	129	105	200	1.78	9.57	0.09	5.22	7.13	3.45
19.2	16.3	486	7.60	8.02	149	88	230	1.07	13.7	0.17	10.5	10.3	5.98
20.1	18.3	487	7.57	7.82	100	100	270	1.57	22.8	0.09	5.13	9.43	6.67

21.1	17.6	388	7.76	8.72	107	25	170	0.62	2.14	0.01	2.14	15.9	7.59
21.2	17.6	388	7.76	8.72	107	25	170	0.62	2.14	0.01	2.14	15.9	7.59
21.3	18.2	397	7.04	8.40	110	74	220	0.62	1.80	0.03	3.68	16.1	2.99
22.1	16.7	305	7.33	8.97	103	25	40	0.50	1.67	0.04	3.93	19.3	5.75
23.1	18	668	8.22	9.87	218	96	240	0.97	30.9	0.05	3.48	5.75	2.07
24.1	17.4	342	7.43	7.70	125	25	130	0.23	9.82	0.04	5.09	15.6	2.99
25.1	17.8	362	7.47	8.27	171	93	170	0.31	5.32	0.04	4.06	14.0	2.53
26.1	19.2	356	6.96	6.18	84	25	95	0.93	4.65	0.05	4.60	26.2	3.91
27.1	19.4	437	7.28	6.86	158	58	190	0.27	5.79	0.04	4.17	12.9	4.83

Table S3. Sediment parameters determined for each sampled sites. Organic matter (OM - % w/w), fine and coarse particles content (% w/w), and Phi benchmarks were included in the sediment subset.

	OM	Fines	Coarse	Phi MODE	Phi MEDIAN	Phi QD
1.1	0.08	0.12	0.00	1.5	1.00	0.38
1.2	0.07	0.07	0.31	0.5	0.25	0.38
1.3	0.08	0.11	0.04	1.5	0.75	0.38
2.1	0.10	0.09	0.83	1.5	0.63	0.50
2.2	0.11	0.12	0.02	1.5	0.75	0.38
3.1	0.10	0.11	0.19	1.5	0.75	0.38
3.2	0.74	1.30	5.87	1.5	1.00	0.44
4.1	0.44	1.26	10.5	1.5	0.63	0.75
5.1	0.17	0.63	0.06	1.5	1.00	0.38
5.2	0.36	2.29	5.14	2.5	1.50	1.00
6.1	0.77	1.57	0.08	1.5	1.00	0.38
7.1	0.40	0.22	0.02	1.5	1.00	0.31
7.2	0.07	0.23	0.05	1.5	1.00	0.38
8.1	1.52	2.47	8.96	1.5	1.00	0.44
9.1	1.65	2.26	2.96	1.5	0.88	0.50
10.1	0.19	0.22	0.25	1.5	0.75	0.50
11.1	0.33	0.59	0.14	1.5	1.00	0.25
11.2	0.94	0.24	0.04	1.5	0.63	0.50
11.3	0.12	0.19	3.39	1.5	0.75	0.44
12.1	3.07	2.64	0.44	1.5	0.88	0.38
13.1	1.94	1.03	0.01	1.5	0.88	0.38
14.1	0.29	0.59	1.52	1.5	0.75	0.56
14.2	0.13	0.46	0.33	1.5	1.00	0.25

15.1	0.46	1.31	0.52	1.5	1.00	0.44
15.2	5.63	11.6	29.0	1.5	0.75	1.94
16.1	0.12	0.23	0.73	1.5	0.75	0.56
17.1	4.20	16.5	0.32	2.5	1.75	0.69
18.1	0.18	0.52	0.88	1.5	0.75	0.50
19.1	0.12	0.51	0.29	1.5	0.75	0.50
19.2	0.12	0.41	0.74	1.5	1.00	0.31
20.1	0.26	0.30	0.02	1.5	1.13	0.38
21.1	0.16	0.32	0.00	1.5	1.13	0.38
21.2	0.16	0.39	62.5	-2.5	-2.50	1.88
21.3	0.21	0.37	5.14	1.5	0.75	0.56
22.1	0.10	0.15	3.35	0.5	0.25	0.50
23.1	0.43	1.49	0.29	1.5	1.00	0.38
24.1	0.59	1.07	0.59	1.5	0.75	0.50
25.1	0.09	0.25	0.07	1.5	0.63	0.56
26.1	0.32	0.77	0.00	1.5	1.25	0.50
27.1	0.24	1.54	7.12	0.5	0.25	0.69

Table S4. Hydromorphology characterization of the sampled sites. Four continuous variables were recorded: depth (m), water width (Wwid - m), channel width (Cwidth - m) and flow (m s^{-1}) Emerging, floating and filamentous macrophytes (EmeM, FloM and FilM, respectively), as well as filamentous algae (FilA) were recorded as present (1) or absent (0); the extent of water shading (shade) felt in each site was quantified under a discrete scale of 4 levels (0 = none; 1 = < 30%; 2 = 30-60%; 3 = > 60%), and riparian vegetation density in the right and the left banks (RBrip and LBrip, respectively) was quantified under a discrete scale of 5 levels (0 = none; 1 = isolated/scattered; 2 = occasional clumps; 3 = semi-continuous; 4 = continuous).

	Depth	**Wwid**	**Cwid**	**Flow**	**EmeM**	**FloM**	**FilM**	**FilA**	**Shade**	**RBrip**	**LBrip**
1.1	0.45	15.0	18.0	0.444	1	0	0	0	1	0	0
1.2	0.20	15.0	18.0	0.097	0	0	0	0	1	0	0
1.3	0.40	15.0	18.0	0.097	0	0	0	1	1	0	0
2.1	0.70	2.2	2.2	0.028	0	0	0	0	1	0	0
2.2	0.65	25.0	25.0	0.042	1	0	0	1	1	0	0
3.1	0.60	5.0	7.0	0.081	1	0	0	1	2	0	0
3.2	0.50	5.0	8.0	0.138	1	0	0	0	3	0	0
4.1	0.40	2.5	15.0	0.139	1	0	0	0	3	1	1
5.1	0.30	4.0	6.0	0.039	0	0	0	0	1	4	4
5.2	0.30	4.0	5.0	0.039	0	0	0	0	2	4	4

6.1	0.10	2.0	3.0	0.047	0	0	0	1	2	4	4
7.1	0.50	30.0	30.0	0.004	1	0	0	0	2	0	0
7.2	0.60	30.0	30.0	0.100	1	0	0	0	2	0	0
8.1	0.80	6.0	8.0	0.000	0	1	1	1	0	4	4
9.1	0.10	1.5	2.0	0.039	0	0	0	1	2	4	4
10.1	0.40	16.0	20.0	0.114	0	0	0	0	1	4	4
11.1	0.75	23.0	25.0	0.108	0	0	0	0	3	4	0
11.2	0.60	25.0	25.0	0.078	0	0	0	0	2	0	0
11.3	0.60	25.0	28.0	0.106	1	0	0	0	2	3	4
12.1	0.15	4.0	4.0	0.036	0	0	0	0	1	4	3
13.1	0.60	25.0	30.0	0.007	0	0	0	0	1	4	4
14.1	0.35	30.0	30.0	0.092	0	0	0	0	1	0	0
14.2	0.30	30.0	30.0	0.022	1	1	0	0	1	0	0
15.1	0.30	7.0	7.0	0.064	0	0	0	1	0	0	0
15.2	0.60	15.0	15.0	0.000	0	0	0	1	2	0	4
16.1	0.30	8.0	8.0	0.139	0	0	0	0	3	1	0
17.1	0.35	5.0	5.0	0.031	0	0	0	0	2	3	1
18.1	0.65	8.0	8.0	0.000	0	0	0	1	2	0	0
19.1	0.35	10.0	10.0	0.108	1	0	0	0	3	1	1
19.2	0.75	15.0	15.0	0.076	1	0	0	0	2	2	2
20.1	0.30	2.5	2.5	0.075	0	0	0	0	3	0	0
21.1	0.20	5.0	5.0	0.028	0	0	0	0	3	2	2
21.2	0.56	5.0	5.0	0.167	0	0	0	0	3	2	2
21.3	0.60	20.0	20.0	0.039	1	0	0	0	3	1	2
22.1	0.40	6.0	6.0	0.094	0	0	0	0	3	4	4
23.1	0.33	6.0	8.0	0.031	0	0	0	1	3	0	1
24.1	0.70	10.0	10.0	0.175	1	0	0	0	3	3	1
25.1	0.40	7.0	7.0	0.133	0	0	0	0	3	4	4
26.1	0.35	2.0	3.0	0.097	1	0	0	0	3	0	3
27.1	0.40	6.0	14.0	0.094	0	0	0	1	1	1	1

Table S5. Biotic subset composed of 7 variables calculated on the basis of the benthic macroinvertebrate communities assessed in all sampling sites. Total macroinvertebrate abundance, macroinvertebrate diversity (H'), and equitability (J') were calculated, as well as 4 biotic indices: Ephemeroptera, Plecoptera, Trichoptera (EPT) index; Iberian Biological Monitoring Working Party (IBMWP) index; Iberian Average Score per *Taxon* (IASPT) index; South Invertebrate Portuguese (IPtIs) index.

	Abundance	H'	J'	EPT	IBMWP	IASPT	IPTIs
1.1	3	0.00	0.00	0	0	0.00	0.00
1.2	24	0.79	0.72	0	5	2.50	0.09
1.3	59	1.16	0.56	1	25	3.57	0.27
2.1	44	1.21	0.67	1	18	3.60	0.23
2.2	65	1.25	0.78	1	14	3.50	0.25
3.1	87	1.18	0.57	1	23	3.29	0.29
3.2	322	1.60	0.62	3	50	4.17	0.52
4.1	86	1.27	0.58	1	30	3.75	0.34
5.1	265	0.98	0.50	2	27	3.86	0.33
5.2	335	1.12	0.45	3	57	4.75	0.49
6.1	623	0.81	0.31	2	49	3.77	0.45
7.1	198	1.49	0.72	3	28	4.67	0.38
7.2	80	1.11	0.54	1	18	3.00	0.24
8.1	335	1.18	0.42	1	62	3.88	0.47
9.1	3378	1.00	0.42	2	41	4.10	0.41
10.1	8	0.90	0.82	1	11	3.67	0.18
11.1	114	1.40	0.67	3	28	4.00	0.38
11.2	41	0.92	0.57	1	11	3.67	0.22
11.3	206	1.33	0.52	4	46	4.18	0.48
12.1	684	1.08	0.47	3	35	4.38	0.41
13.1	269	0.41	0.20	3	31	4.43	0.39
14.1	65	1.44	0.56	2	46	4.18	0.45
14.2	138	1.17	0.53	2	28	3.50	0.31
15.1	2560	0.32	0.13	4	41	4.56	0.46
15.2	385	1.07	0.51	2	22	3.14	0.27
16.1	48	0.60	0.37	1	13	3.25	0.19
17.1	45	0.29	0.26	0	8	4.00	0.17
18.1	340	0.49	0.27	2	19	3.80	0.27
19.1	228	1.63	0.63	6	56	4.67	0.66
19.2	382	1.97	0.70	4	69	4.31	0.72
20.1	170	1.08	0.47	2	30	3.75	0.41

21.1	65	1.56	0.68	3	40	4.44	0.47
21.2	1134	1.14	0.47	3	49	4.90	0.60
21.3	105	1.42	0.62	2	49	5.44	0.51
22.1	48	1.46	0.61	3	57	5.70	0.57
23.1	2909	0.14	0.06	3	38	4.22	0.45
24.1	95	1.84	0.74	3	39	3.90	0.47
25.1	11	1.26	0.91	0	12	3.00	0.14
26.1	979	1.88	0.64	4	67	3.94	0.62
27.1	507	1.26	0.65	1	16	2.67	0.20

Table S6. Codes (in bold) used for plotting macroinvertebrate taxa (Figure 5).

Aes	Aeshnidae	**Hir**	Hirudinea
Ant	Antomyiidae	**Hydb**	Hydrobiidae
Ase	Asellidae	**Hydme**	Hydrometridae
Ath	Athericidae	**Hydph**	Hydrophilidae
Aty	Atyidae	**Hydps**	Hydropsychidae
Bae	Baetidae	**Hydpt**	Hydroptilidae
Ber	Beraeidae	**Leu**	Leuctridae
Cae	Caenidae	**Lib**	Libellulidae
Cal	Calopterygidae	**Lim**	Limnephilidae
Cer	Ceratopogonidae	**Lim**	Limoniidae
Chi	Chironomidae	**Oli**	Oligochaeta
Coe	Coenagrionidae	**Phr**	Phryganeidae
Cor	Corixidae	**Phys**	Physidae
Dry	Dryopidae	**Pla**	Planorbidae
Dys	Dysticidae	**Plat**	Platycnemididae
Ecn	Ecnomidae	**Psy**	Psychodidae
Elm	Elmidae	**Psym**	Psychomyiidae
Emp	Empididae	**Pup**	Pupas
Ephl	Ephemerellidae	**Rya**	Ryacophilidae
Ephr	Ephemeridae	**Sai**	Sialidae
Erp	Erpobdellidae	**Sim**	Simulidae
Ger	Gerridae	**Tip**	Tipulidae
Glop	Glossiphoniidae	**Val**	Valvatidae
Glom	Glossosomatidae	**Pla**	Planorbidae
Gom	Gomphidae	**Plat**	Platycnemididae
Gyr	Gyrinidae	**Psy**	Psychodidae

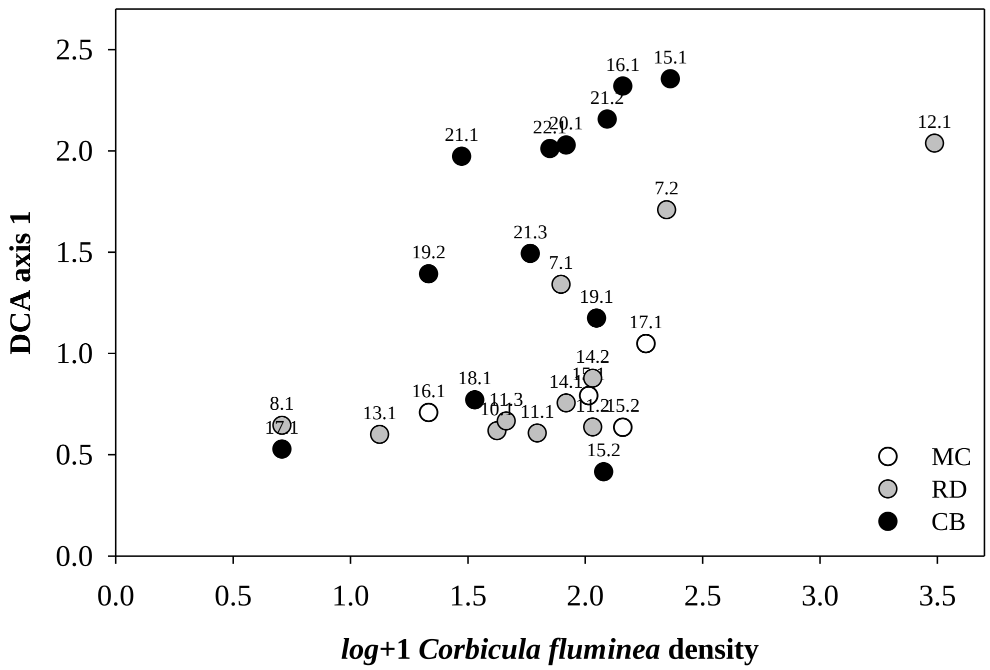


Fig S1. Relationship between clam density (*log*+1 density) and macroinvertebrate community structure (axis 1 scores of the DCA ordination) in invaded sites. The artificially defined systems within the catchment area (MC – Mira channel; RD – Real ditch; CB – Corujeira brook) were identified in grey scale for clarity purposes. Sites were identified immediately above the corresponding symbol.

CAPÍTULO V

Photographic key for the identification of Portuguese freshwater macroinvertebrates: a tool for societal involvement within the Water Framework Directive perspective

Abstract

The European Water Framework Directive (WFD) brought the need in European Union countries to establish consistent quantitative methods for the water quality assessment of streams. Societal involvement in environmental issues is also a critical flag of the European policy nowadays. In this way, the development of suitable tools allowing the society to get enrolled in the conservation principles behind the goals of the WFD is a current need. The use of benthic macroinvertebrates as indicators of the ecological status of lotic systems is advantageous in several ways, but difficulties in the taxonomical classification of these organisms may constrain the accessibility to the methodology by non-specialist audiences. Photographic dichotomous keys are tools that can assist the overcoming of such a drawback, making benthic macroinvertebrates identification an easier task for anyone interested. In this study, photographic (3D) identification key for freshwater macroinvertebrates found in Portuguese (also in Iberia in general) lotic systems was developed and validated. A dichotomous structure was selected, based on easily distinguishing features, to facilitate reliable, family-level identification of collected specimens. This key was validated with two distinct groups of Higher Education students (within and out of biological sciences fields). The evaluation was conducted through a questionnaire applied immediately following contact with the photographic key for the classification of a reference collection of preserved macroinvertebrates. Results showed that both validation groups correctly identified the reference organisms, even though most of the students had no previous experience at all in the observation and identification of benthic macroinvertebrates. Thus, the new key can be a handy tool to support Environmental Education actions within the preservation of the lotic ecosystems.

Key Words: WFD, water quality, dichotomous keys, macroinvertebrates identification, societal involvement.

Introduction

Throughout Europe, streams have experienced a long history of modification by humans and have become amongst the most threatened ecosystems worldwide (Marzin et al., 2012). Since the WFD was enforced, integration is a key word in the field of water policy in Europe and a main goal for decision makers involved in the process of river basins assessment and management (Gottardo et al., 2011). As stated by the European Commission (EC, 2003), integration concerns different elements and steps of the WFD implementation, starting from the setting up of multiple environmental objectives (including aquatic ecosystems and water quality) up to the involvement of stakeholders and the civil society in decision making. Although the technical implementation of WFD purposes is a complex process, the use of several quality elements and establishment of ecosystem typologies and reference conditions is a major improvement in the water system protection (Hering et al., 2010). In this context, the WFD requires that countries assess the 'ecological status' of their freshwater bodies using three quality elements: biological, physicochemical and hydromorphological status. The evaluation of the freshwater biological status was recognized as being a much more effective as an integrative way to measure ecological quality (Hering et al., 2010), since the diversity of communities and organisms occurring in aquatic environments reflects the overall structure and functioning of the ecosystems (Gottardo et al., 2011). Biological status is based on assessment of specific aquatic assemblages, termed biological quality elements (BQEs), namely fish, invertebrates, diatoms, plants and phytoplankton. Among BQEs, benthic macroinvertebrates have a long history as biomonitoring tools, being the most widely used biological group in freshwater bioassessment (Mondy, Villeneuve, Archaimbault, & Usseglio-Polatera, 2012).

Several features have lead to the touting of these organisms as major biomonitoring tools. Benthic macroinvertebrates used as BQEs can be seen even without the aid of a microscope (Ancog, Andrade, Miasco, & Ortiz, 2010). They usually inhabit bottom substrates (e.g. sediments, debris, logs, macrophytes, filamentous algae) of freshwater *habitats*, for at least part their life cycle (Rosenberg & Resh, 1993). These organisms feed largely on algae, bacteria and organic matter (Ancog et al., 2010), being important in the energy transfer throughout aquatic food webs (Abde & Alemayehu, 2014). In this context, macroinvertebrate communities play a vital role in the transfer of energy from primary producers to fish (Abde & Alemayehu, 2014), which are their most likely predators. Rosenberg & Resh (1993) claim that while the abundance, diversity and structure of macroinvertebrate communities may be relatively stable under constant environmental conditions, they respond to changes and, as such, are often used as bioindicators of ecosystem change.

Usually, macroinvertebrates are used as bioindicators by professional streams ecologists and they are identified to the lowest practical taxonomical level, generally the family (or genus, when possible) using standard keys (Macan, 1959; Elliott, 1977; Pattée & Gourbault, 1981; Richoux, 1982; Tachet, Bournaud, & Richoux, 1980; Sundermann, Lohse, Beck, & Haase, 2007; Serra, Coimbra, & Graça, 2009). These macroinvertebrate identification keys generally use graphics of distinguishing features (e.g. body shape, location of prolegs, presence and location of gills and tails, the shape of the head capsule). Even though these keys are detailed, experience and expertise is often required to interpret the indications given. A consistent theoretical background on the species morphology is required, and relative measurements are often used (e.g. paraglossa is higher than glossa) that difficult judgement by non-expert individuals.

In spite of these expertise requirements, bioassessment professionals can increase their capacity to understand and manage local ecosystems if they work with residents who live in, and interact with, local ecosystems (Nerbonne & Nelson, 2008). Moreover, exposing scientific information to citizens can also help in involving them in citizen science campaigns; this was already evidenced e.g. by monitoring projects examining changes in species distribution and composition in the short-, medium- and long-period (Martellos & Nimis, 2015). This involvement of citizens in monitoring lotic ecosystems, as well as their participation in the development of effective environmental policies, is important for an overall understanding of the value of biodiversity (Jiménez, Díaz, Monroe, & Benayas, 2014). Schusler, Decker, & Pfeffer (2013, p.311) refer that when ‘people engage one another, sharing diverse perspectives and experiences to develop a common framework of understanding and a basis for joint action’ social learning occurs.

Therefore, through planning partnerships with citizen organizations and others in collaborative conservation initiatives, natural resource professionals and citizens can increase their capacity to protect local ecosystems by sharing resources, promoting a shared understanding, and ultimately working together to effectively influence environmental policy (Norton, 2003). In this context, suitable (i.e. of easy understanding and interpretation, as well as visually engaging) tools that can be used by non-expert individuals should be built and made available.

The present work intended to develop and validate a visually engaging and simplified dichotomous key to identify Portuguese benthic macroinvertebrates that could be used by a broad public with residual scientific knowledge on stream ecology. This key is supported by conspicuous photographic reports of essential identification details, and 3D edition was used to benefit the clarity of these reports and improve the visual attractiveness. This allows a rigorous use of the keys, but still facilitating it, hence encouraging the use by students, and people who are taking the first steps into the world of freshwater macroinvertebrates identification. The use of maximum detail at relatively low levels of taxonomical identification (i.e. Family levels) was a clear option from the beginning. It has the advantages of facilitating the recognition of intended features, preventing errors and differential identification outcome by different users, ultimately favouring the biological assessment performance (De Pauw & Vanhooren, 1983).

Materials and Methods

Macroinvertebrate collection and photographic documentation

Benthic macroinvertebrates, used for the construction of the identification key, were selected after a literature search that focused on those typically found in streams of Portugal (e.g. INAG, 2008; Serra et al., 2009) and according to the experience of the research team members about this group of organisms.

Most of the photographs were taken on benthic macroinvertebrates from reference collections existent in the laboratory, resulting from previous collections made in various streams and rivers throughout the country. Still, new samples had to be collected for the present study since some groups are absent from the reference collections and to replace some small specimens whose deficient conservation in the long term lead to substantial modification of shapes and colours. Tridimensional (3D) photograms were widly use to illustrate the key steps; these were built on series of photographs taken with a camera (Olympus SC30) coupled to a stereoscope (Olympus SZX9), through a dedicated software (HeliconSoft). The standard protocol for each 3D photogram was as follows: i) lens were adjusted to capture the closest area of the organism and the first shot was taken; ii) focus of progressively distant images was made followed by a documenting shot until reaching the farthest part of the scene; iii) the various photographs were combined, using the dedicated software and manual adjustments to obtain a single 3D image. In cases where it was not possible to find an adequate specimen to complete the key, the photographs were rendered from the published taxonomic keys and quoted accordingly.

Designing the photographic dichotomous key

A dichotomous key is a sequential series of paired statements about certain group of organisms and is used to identify an unknown specimen. The paired statements describe opposite characteristics. The chosen statement may ask to go on to another pair of statements or it may drive the operator directly towards the name of the organism (Watson-Ferguson, Han, McGarvey, & Miller, 2006). In this way, the preparation of 3D dichotomous keys was based on dichotomous keys already published, which were adapted to the Portuguese reality (communities typically found in Portuguese streams and rivers), thus making them more attractive and easy to use. The photographs used in this key show morphological aspects of the various groups of benthic macroinvertebrates (e.g., body shape, position of prolegs, presence and location of gills and tails, shape of the head capsule). Besides this photographic documentation, certain steps generally present in published specialise keys were eliminated when unnecessary to further facilitate the identification of organisms. For example, in the dichotomous key by Serra et al. (2009), the identification of the Order Plecoptera begins with the characteristic 'size glossa/paraglossa'. Because it is difficult to distinguish glossa and paraglossa, this characteristic was replaced by another distinguishing one, the shape (pointed) of the 'last segment of maxillary palps', which is more easily noticeable to anyone without losing on the reliability of the key.

An omnibus panel divides into 12 keys, one per major macroinvertebrate groups, allow the identification within the group until the family level by someone hypothetically lacking specialised knowledge in systematics. Either adults or larvae were considered in the keys, depending on the prevalence of each stage within Portuguese macroinvertebrate communities.

Key validation

The validation of the key was conducted with 36 volunteers from University of Aveiro and Polytechnic Institute of Viseu – Higher Education context. These volunteers were divided into two groups: one group collecting individuals with formal training in biological sciences (G1, N=18) and another collecting individuals with no formal training in biological sciences (G2, N=18). The composition of the sample within each group was carefully designed to cover different levels of higher education and, within the group with training in biological sciences, different levels of understanding on the ecology of benthic macroinvertebrates (Table 1).

Table 1. Detailed composition of the sample within each group. The stage of higher education of the individuals is shown in the first column and then the specialisation area is clarified for G1, with individuals bearing formal training in biological sciences, and G2, with individuals bearing no such training.

	G1	G2
1st year degree	Environmental Education Basic Education	Cultural Animation Advertising and Public Relations
3rd year degree	Environmental Education Basic Education	Social Education Advertising and Public Relations
Postgraduate	MScs in Biology, Toxicology and Ecotoxicology, Coastal Zones Sciences, Management and Environmental Policy, Didactic Sciences PhD in Biology	Graduated in Social Education MScs in Health Management Units PhD in Chemical Engineering

The key validation was appointed at the convenience of the volunteers, who immediately received general instruction on how to use a dichotomous keys and how to use a stereoscope. The identifications of specimens provided were conducted individually, with the aid of a stereoscope and on the basis of a paperback version of the developed photographic identification key. All volunteers were given a previously prepared sample containing three specimens belonging to different families for identification. The three specimens were combined to qualitatively range the identification job from easy to difficult within the sample: a specimen belonging to the Order Diptera (Family Chironomidae) was set as of easy identification; a specimen belonging to the Order Trichoptera (Family Limnephilidae) represented an average level identification; and a specimen belonging to the Order Plecoptera (Family Perlodidae) was selected to represent a difficult level identification.

This activity lasted on average for one hour and when all identifications were completed volunteers filled out a questionnaire (see supplementary material). This questionnaire, applied to all volunteers involved in the present study, was used in a first stage for data collection regarding the characterization of the study sample, its previous level of knowledge on benthic macroinvertebrates and on the use of dichotomous keys. In a second stage, the questionnaire aimed at assessing the suitability of the photographic key for macroinvertebrate identification, through the verification of the correct identification of the three tested organisms and the classification on the easiness of the key use.

Data were collected on the basis of responses to survey questions. For each question, the frequency of response categories was calculated for descriptive data analysis. A Chi-Square Test (χ^2) was used to test the independence between the rows and columns of a contingency table which crosses two nominal variables, including whether to verify the existence of an association. In this study, χ^2 was used to distinguish the responses of groups 1 and 2 in questions 1, 2 and 3 of part II of the questionnaire (H_0: There is no significant association between the responses of the G1 and G2; H_1: There is a significant association between the responses of the G1 and G2). The χ^2 only provides reliable results if the percentage of contingency table cells, with the expected frequency less than 5, is less than 20%. Otherwise, for a 2x2 table, instead seeing the result on Pearson Chi-Square line, we should read it on Fisher's Exact Test line, because this test adjust the results in the presence of violation of this assumption (Martins, 2011). All statistical analyses were produced using SPSS 22, setting the confidence interval to 95% and defining the significance level of the study (p-value) at 0.05.

Results and Discussion

While allowing more rigorous identification, the use of dichotomous keys, published in specialist works, does not always produce reliable results due to the fact that the majority of available keys are not completely adapted to the Portuguese reality, including species that do not occur in our lotic ecosystems.

The use of such dichotomous key for storing and managing the water quality is no longer limited to an academic audience (Martellos & Nimis, 2015). It can be also a relevant tool in other social arenas where the interest towards nature and the environment is shared, e.g. to amateurs, tourists, or citizen scientists that desire and can acceded to new knowledge, and then eventually share it. Most of the macroinvertebrates-related resources published, for example, on the web are potentially accessible to a wide audience, but their user-friendliness is often limited (Martellos & Nimis, 2015). Identification tools should be designed in such a way that an optimal balance between usuability and feasible is reached (Martellos & Nimis, 2015). Moreover, keys for non-experts should not be simplistic or un-technical, so as not to develop scientifically incorrect conceptions. These, known as alternative conceptions, are ideas that appear as alternatives to scientific versions accepted and they can´t be seen as distractions, memory lapses or miscalculations, but as potential explanatory models resulting from a conscious effort of theorizing (Cachapuz, 1995). Alternative conceptions may arise from distorted or simplified presentations of certain issues, which lead to misunderstanding of individuals (Carrascosa, 2005; Pozo & Crespo, 2006). In addition, they are very resistant to change, and if not identified and deconstructed, they will act as barriers to the knowledge construction (Furió, Solbes, & Carrascosa, 2006).

Designing the photographic dichotomous key

The developed key includes the main macroinvertebrate groups that can be found in Portugal streams (see supplementary material). Despite present within benthic communities, classes Turbellaria and Oligochaeta were not considered for the development of family keys (Figure 1) since the identification to this taxonomical level is not required in stream biomonitoring *sensu* WFD. In addition, some characteristics of the class Oligochaeta (e.g. dorsal bundles) are very small and difficult to observe, requiring the use of a microscope for its correct identification.

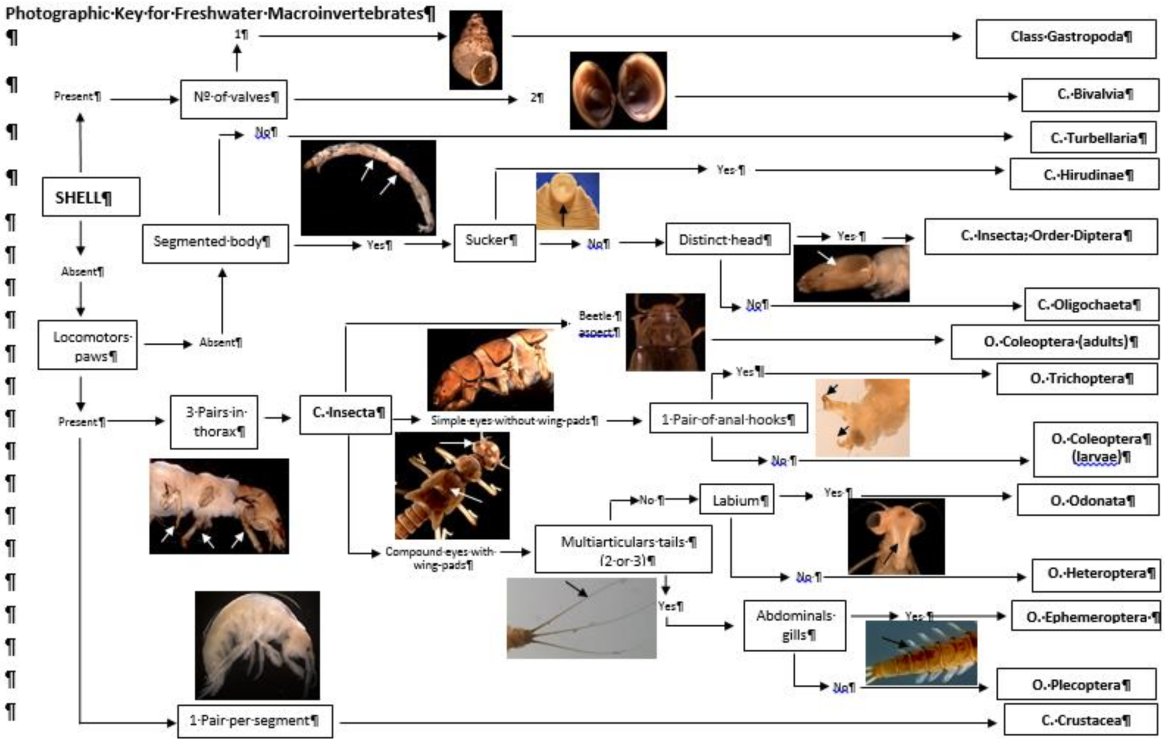


Fig. 1. Omnibus panel of the photographic (3D) dichotomous key for benthic macroinvertebrates identification, developed on the basis of typical groups found in Portuguese streams and rivers.

The construction of dichotomous keys was a long process due to the difficulty to simplify existing keys. The kind of characters that could be eliminated or summarized without promoting identification errors by a non-specialist user were analysed iteratively before assumed in the final version of the key. Some difficulties were also experienced during the photographic documentation of the specimens. On the one hand, preservation deteriorates the quality of the sample, making it difficult the documentation of some structures; on the other hand, the production of 3D photograms requires some degree of expertise for capturing the appropriate images resulting in an adequate assembly towards building 3D model.

By enhancing basic dichotomous key with 3D photographs better orientation is given to the user as to the recognition of the intended distinguishing features in a first observation; drawings typically used in existent keys are frequently hard to interpret without previous specialised knowledge on the organism's morphology (Vollbrecht, Rush, & Cottenie, 2013). Also, by focusing on local *taxa* rather than on *taxa* that users would never find in their activitie, immediate recognition of the organisms is promoted, which favours positive emotions towards the biota and better engagement to its environmental protection ((Stagg, Donkin, & Smith, 2014). Finally, removing species level characters hard to recognise by non-specialised users directly improves the probability of a well succeeded identification that was made easier (Vollbrecht et al., 2013).

Key validation

The questionnaire results generally confirm that the rules established to assembly the opposing groups (Table 1) were met. In spite of the confirmation on a previous background in Biological Sciences as described above, a surprising percentage (40%) of G1 members never had observed macroinvertebrates under the stereoscope (question 5; questionnaire – Part I). These were those members who are at first higher education stages (1st year of a degree in biological sciences). As expected, none of G2 members had previous contact with macroinvertebrate samples under the stereoscope. Both G1 and G2 were largely inexperienced in the identification of benthic macroinvertebrates (61% and 100%, respectively; question 6; questionnaire – Parte I). Although most of G1 members had already observed macroinvertebrates under the stereoscope, only 39% recognise themselves as having some experience in the identification of these organisms within classroom and research projects contexts.

Almost all volunteers composing G1 and G2, were able to correctly identify the three organisms: organism 1 - F. Chironomidae (Figure 2); organism 2 – F. Limnephilidae (Figure 3); organism 3 – F. Perlodidae (Figure 4).

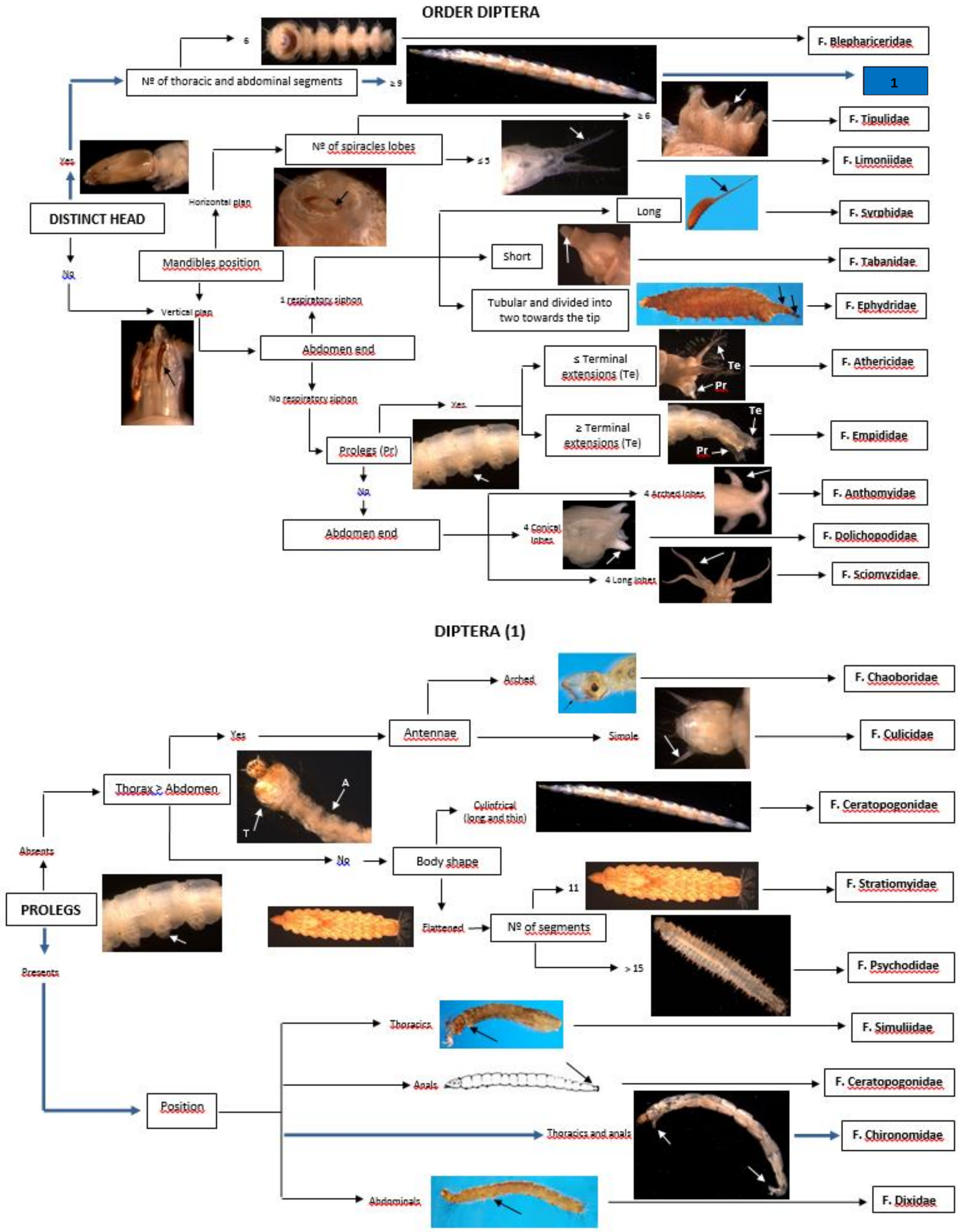


Fig. 2. Dichotomous key for Diptera order identification. The blue colour highlights the way, with easily observable characteristics, leading each volunteer to the correct identification.

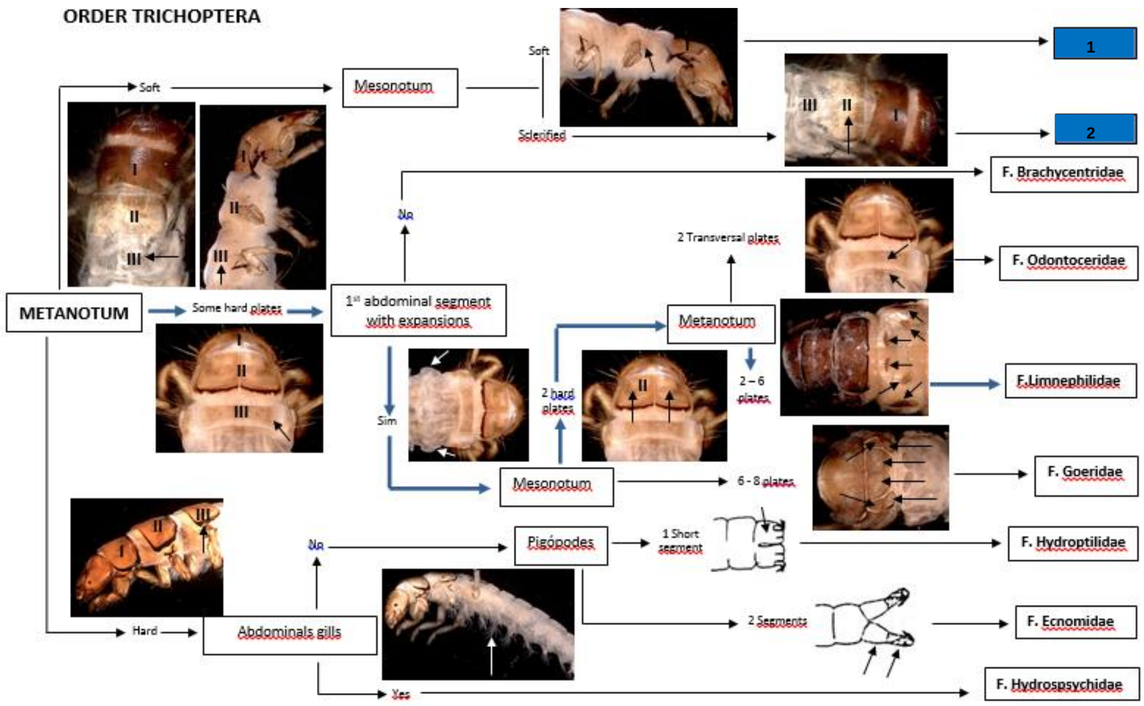


Fig. 3. Dichotomous key for Trichoptera order identification. The blue colour highlights the way, with some of the most difficult characteristics to identify (metanotum and 1st abdominal segment with expansions), leading each volunteer to the correct identification.

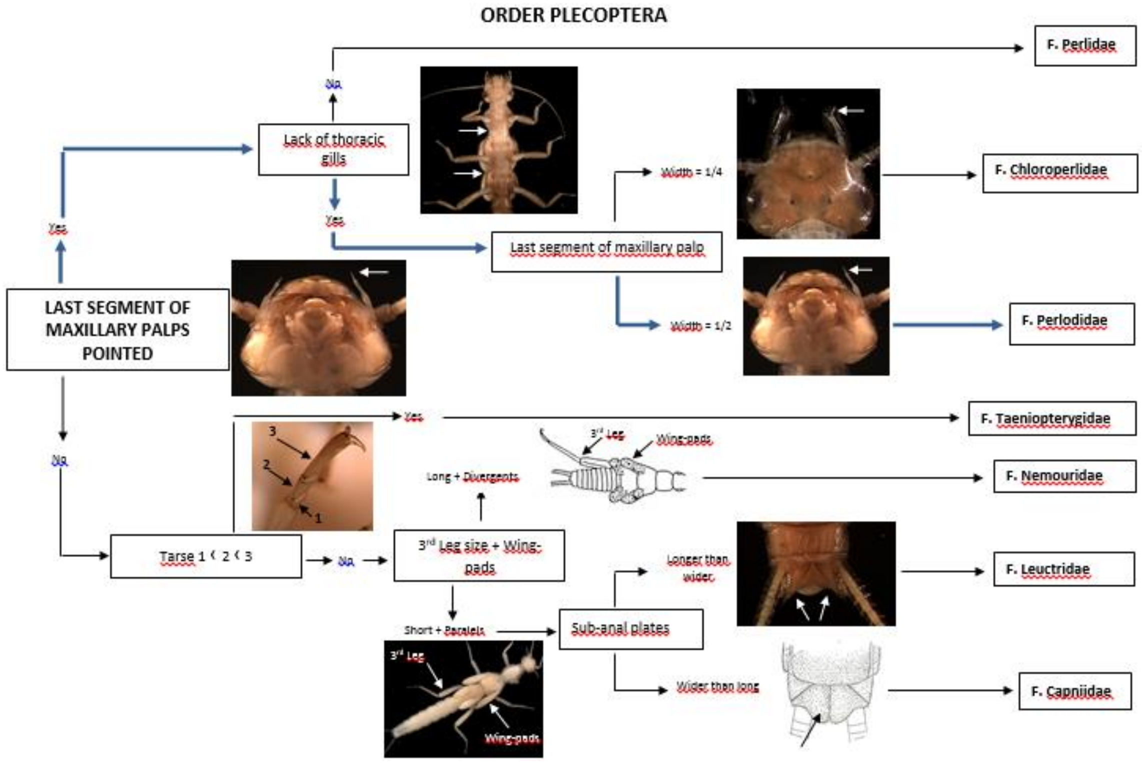


Fig. 4. Dichotomous key for Plecoptera order identification. The blue colour highlights the way, with very small features, difficult to observe, leading each volunteer to the correct identification.

The percentage of both groups failing a correct identification of organism 2, which was selected to represent an average identification, was expected to be lower than that of failure in identification of organism 3, representing a difficult identification. The opposite was found, with organism 2 collecting 33 and 39% failed identifications, and organism 3 collecting 6% and 11% failed identifications, in G1 and G2, respectively (question 1; questionnaire – Part II; Figure 5). It should be noted that the dichotomous key relative to organism 3 is slightly shorter (only 3 steps) than the other two, which may have biased the scaling of difficulty established a priori and consequently the results of the questionnaire. However, there was no significant association between the groups in the correct identification of the organism 1 ($p = 0.66$, Fisher's Exact Test line), organism 2 ($\chi^2 = 0.12$; $p = 0.73$) and organism 3 ($p = 1.0$, Fisher's Exact Test line). This suggests that who had training in biological sciences did not have an advantage in identification ability compared to those who had not.

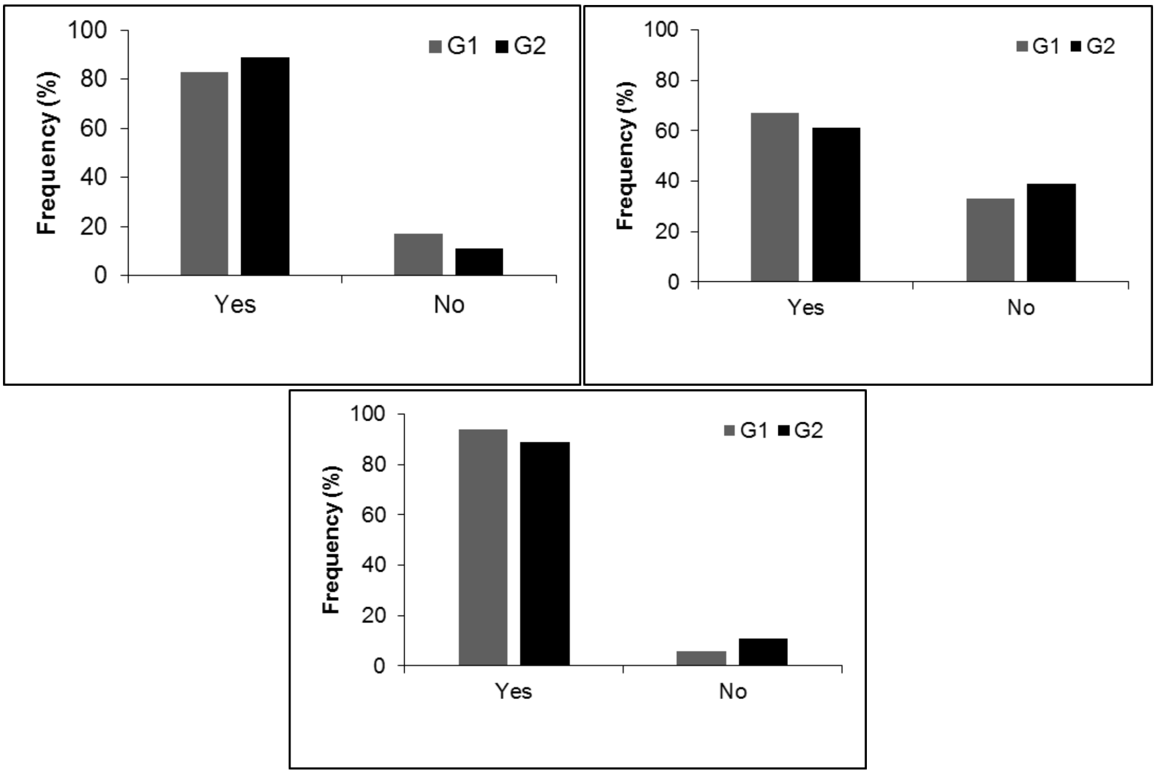


Fig. 5. Frequency of positive (xx' category 'Yes') and negative (xx' category 'No') answers by members of G1 and G2, regarding the correct identification of organism 1, 2 and 3, respectively.

In general, the two groups easily used the photographic dichotomous key in the identification of all organisms, in a sequenced way (question 2; questionnaire – Part II; Figure 6). Contrarily to what was expected, these results confirm that both groups found the key for organism 2 (set up as an average identification) more difficult to use than that provided for the identification of organism 3 (set up as a difficult identification). None of the volunteers had difficulties in using the key for the identification of organism 3. Once again, there was no significant association between the groups in the use of the key for the identification of the organism1 (p = 1.0, Fisher's Exact Test line), organism 2 (χ^2 = 0.12; p = 0.73) and organism 3 (no statistics were calculated, because this parameter is constant – 100%).

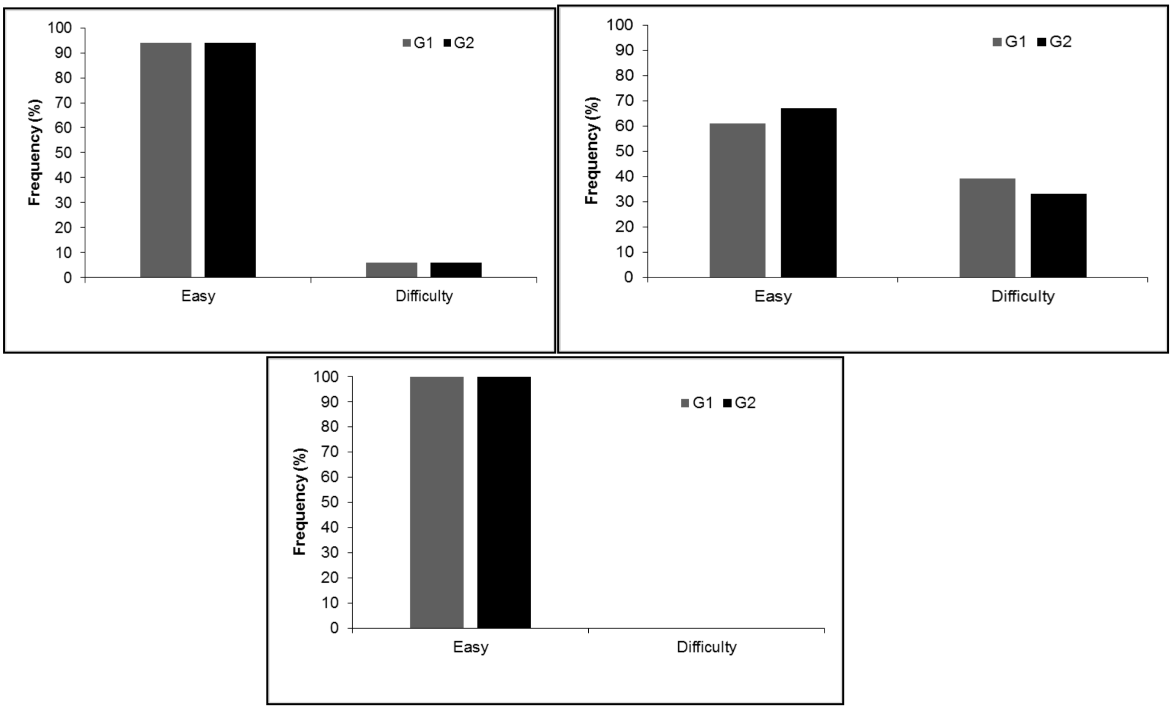


Fig. 6. Frequency of answers (xx' category 'Easy'; xx' category 'Difficult') by members of G1 and G2 when questioned on whether they sequentially use the dichotomous keys for organism 1, 2 and 3, respectively.

In the 'easy identification' category, G1 described the photographic dichotomous key as being 'easy to use/understand' and some users declared that 'some descriptions between characters were clear'. Others comments included: photos were 'helpful/useful', 'very elucidative', 'better than a drawing' and were a 'representation of reality'. G2 made generally the same comments and attributed difficulties to ‘inexperience in the interpretation of dichotomous keys’ and the ‘lack of knowledge on some concepts’ (e.g. prolegs, metanotum, wing-pads).

Interestingly, despite the two groups easily used the photographic key in a sequenced way, they had difficulties in at least one of the key steps (question 3; questionnaire – Part II). For the first organism, G1 and G2 users who had difficulties (28% and 50%, respectively) highlighted mostly the ‘identification of prolegs’ (present/absent), which is the first step of the key. In the case of organism 2, users referred difficulties (50% each group) in the ‘identification of anal hooks and the metanotum’. In contrast, most of them had no difficulty for the organism 3 and those who had it (22% G1 and 33% G2) referred the width of the last segment of maxillary palps (last step of the key). The Chi-Square Test showed, again, that there was no significant difference between the groups in the difficulties felt by volunteers for the organism 1 ($\chi^2 = 1.87$; $p = 0.17$), organism 2 ($\chi^2 = 0.00$; $p = 1.0$) and organism 3 ($\chi^2 = 0.55$; $p = 0.46$).

The correct identification of the tested organisms, through the use of the new key, was probably linked to its presentation format, which allows easy viewing of similar species and differentiating characters. This has been corroborated by the opinions given by volunteers in the written questionnaire (see above). In fact, Lawrence & Hawthorne (2006) claim that usability of an identification guide is determined by the navigability of the key, ease of understanding of the terminology and pictorial information (photos, illustrations, symbols), and ease of location and recognition of differentiation cues in the focal specimens.

Interestingly, the organism 3 was correctly identified by almost all volunteers, despite it´s a priori setting as that bearing the most difficult identification. Some of them told that its identification, through the key was easier because it was the last one to be tested and they were more familiar with the procedure, particularly in the handling the stereoscope and the key interpretation. Previous experience in handling dichotomous keys regardless their format may contribute to the rate of positive identifications (Stagg et al., 2014). Randler & Birtle (2008) also demonstrated that students’ performance with an unfamiliar key was improved if they were first acclimated by using a key based on a familiar group of species or objects, since this reduces the cognitive load associated with key use. Some of volunteers also said that organism 3 was in a better preservation condition (they named 'quality') than organism 2 and that almost all its characteristics could be observed perfectly, which may also facilitated the identification.

The most common criticisms of this new key in written questionnaire are related to difficulty of understanding some character descriptions or using them in differentiation. For example, the characters 'prolegs' and 'anal hooks' would have benefited from the inclusion of additional photographs with another shapes. An attached glossary with many terms, which the volunteer is unlikely to know, would be also useful. In this context, the photographic dichotomous key developed in this study should not be regarded as a finished product, but rather as basis for developing an updated tool, primarily by introducing more and better photographs in steps where this was no possible so far, and then by continuously adapting to the needs and capacities of the users, as well as to their geographic context. Comments, suggestions and difficulties, from volunteers, could contribute over time to become this key a learning resource even more effective in the context of Environmental Education for the acquisition of knowledge, dissemination and preservation of lotic ecosystems.

References

Abde, M., & Alemayehu, E. (2014). Benthic invertebrates diversity and relative abundance in boye pond, Jimma zone South Western Ethiopia. *Journal of Biological and Chemistry Research*, 31 (2), 723-729.

Ancog, R., Andrade, G.B., Miasco, R.B., & Ortiz, M.F. (2010). Water quality and diversityof macroinvertebrate species in rivers of Cebu City, Philippines. *The Philippine Scientist*, 47, 27-45.

Cachapuz, A. (1995). O ensino das Ciências para a excelência da aprendizagem. In A. D. Carvalho (Ed.), *Novas metodologias em Educação*, pp. 349-385. Porto: Porto Editora.

Carrascosa, J. (2005). El problema de las concepciones alternativas en la actualidad (parte I). Análisis sobre las causas que la originan y/ o mantienen. *Eureka sobre Ensinanza y Divulgación de las Ciencias*, 2, 183-208.

De Pauw, N., & Vanhooren, G. (1983). Method for biological quality assessment of watercourses in Belgium. *Hydrobiologia*, 100, 153-168.

EC (2003). *Common implementation strategy for the Water Framework Directive (2000/60/CE)*. *Guidance Document n. 11. Planning process*. Luxembourg: Office for Official Publications of the European Communities.

Elliott, J.M. (1977). *A key to British freshwater Megaloptera and Neuroptera*. Ambleside: Freshwater Biological Association.

Furió, C., Solbes, J., Carrascos, J. (2006). Las ideas alternativas sobre conceptos científicos: três décadas de indestigación. *Alambique*, 48, 64-77.

Gottardo, S., Semenzin, E., Giove, S., Zabeo, A., Critto, A., de Zwart, D., … Marcomini, A. (2011). Integrated Risk Assessment for WFD Ecological Status classification applied to Llobregat river basin (Spain). Part II — Evaluation process applied to five environmental Lines of Evidence. *Science of the Total Environment*, 409, 4681-4692.

Hering, D., Borja, A., Carstensen, J., Carvalho, L., Elliott, M., Feld CK, … Bund, W. (2010). The European Water Framework Directive at the age of 10: a critical review of the achievements with recommendations for the future. *Science of Total Environmental*, 408, 4007–4019.

INAG (2008). *Manual para a avaliação biológica da qualidade da água em sistemas fluviais segundo a Diretiva Quadro da Água. Protocolo de amostragem e análise para os macroinvertebrados bentónicos*. Lisboa: Ministério do Ambiente, Ordenamento do Território e do Desenvolvimento Regional. Instituto da Água.

Jiménez, A., Dáz, M.J., Monroe, M.C., & Benayas, J. (2014). Analysis of the variety of education and outreach interventions in biodiversity conservation projects in Spain. *Journal for Nature Conservation*, http:// dx.doi.org/ 10.1016/j.jnc.2014.07.002.

Lawrence, A., & Hawthorne, W. (2006). Plant identification - *creating user-friendly field guides for biodiversity management*. London: Earthscan.

Macan, T.T. (1959). *A guide to freshwater invertebrate animals*. London: Longman Group Limited.

Mazin, A., Archaimbaul, V., Belliard, J., Chauvin, C., Delmas, F., & Pont, D. (2012). Ecological assessment of running waters: Do macrophytes, macroinvertebrates, diatoms and fish show similar responses to human pressures? *Ecological Indicators*, 23, 56-65.

Martellos, S., & Nimis, P.L. (2015) From Local Checklists to Online Identification Portals: A Case Study on Vascular Plants. *PLoS ONE*, 10, 1-18.

Martins, C. (2011). Manual de análise de dados quantitativos com recurso ao IBM SPSS: saber decidir, fazer, interpretar e redigir. Braga: Psiquílibrios Edições.

Mondy, C.P., Villeneuve, B., Archaimbault, V., & Usseglio-Polatera, P. (2012). A new macroinvertebrate-based multimetric index (I2M2) to evaluate ecological quality of French wadeable streams fulfilling the WFD demands: A taxonomical and trait approach. *Ecological Indicators*, 18, 452-467.

Nerbonne, J.F., & Nelson, K.C. (2008). Volunteer macroinvertebrate monitoring: Tensions among group goals, data quality, and outcomes. *Environmental Management*, 42, 470-479.

Norton, B. (2003). *Searching for sustainability: interdisciplinary essays in the philosophy of conservation biology*. Cambridge, UK: Cambridge University Press.

Pattée, E., & Gourbault, N. (1981). *Turbellaries triclades paludicoles (Planaires d'eau douce). Introduction pratique a la systematique des organismes des eaux continentales françaises.* Paris: Association Française de Limnologie.

Pozo, J.I., & Crespo, M.A.G. (2006). *Aprender y Enseñar Ciencia*. Madrid: Ediciones Morata.

Randler, C., & Birtle, A. (2008). The ‘System of Pasta’–An Introduction to Dichotomous Keys. *School Science Review*, 90, 1–6.

Richoux, P. (1982). Coleopteres aquatiques (genres: adultes et larves). *Introduction pratique a la systematique des organismes des eaux continentales françaises.* Lyon: Bulletin de la Societé Linnéenne de Lyon.

Rosenberg, D.M., & Resh, V.H. (1993). *Freshwater biomonitoring and benthic macroinvertebrates*. New York, USA: Chapman & Hall.

Schusler, T., Decker, D., & Pfeffer, M. (2003). Social learning for collaborative natural resource management. *Society and Natural Resources*, 15, 309–326.

Serra, S., Coimbra, N., & Graça, M. (2009). *Invertebrados de Água Doce. Chave de Identificação das Principais Famílias*. Coimbra: Imprensa da Universidade de Coimbra.

Stagg, B.C., Donkin, M.E., & Smith, A.M. (2014). Bryophytes for Beginners: The usability of a printed dichotomous key versus a multi-access computer-based key for bryophyte identification. *Journal of Biological Education*, http://dx.doi.org/10.1080/00219266.2014.934900.

Sundermann, A., Lohse, S., Beck, L.A., & Haase, P. (2007). Key to the larval stages of aquatic true flies (Diptera), based on the operational taxa list for running waters in Germany. *Annales De Limnologie-International Journal of Limnology*, 43, 61-74.

Tachet, H., Bournaud, M., & Richoux, P. (1980). *Introduction à l'étude des macroinvertébrés des eaux douces*. Université Lyon: Association Française de Limnologie.

Vollbrecht, L., Rush, M.T., & Cottenie, K. (2013). Improving dichotomous keys for undergraduate teaching. *Studies Undergraduate Researchers at Guelph*, 7, 23-32.

Watson-Ferguson, K., Han, C., McGarvey, J., & Miller, L. (2006). *A guide to aquatic insects & crustaceans*. USA: Stackpole Books.

Supplementary Material

Questionnaire

University/ Polytechnic Institute: ___________________________________

Benthic macroinvertebrates can be seen even without the aid of a microscope. They usually inhabit bottom substrates of freshwater habitats, for at least part their life cycle organisms. These organisms have been widely used to assess the biological quality of lotic ecosystems (water with current; e.g., rivers).

This questionnaire is aimed at validating a macroinvertebrate identification key developed within the PhD of Cristiana Duarte Mendes (University of Aveiro). Thanks for your cooperation by answering the following questions.

Part I

1. Gender: Female ___   Male ___

2. Age: ___

3. Academic qualification:

3.1. To attend a undergraduate degree: 1st year ___ 2nd year ___ 3º year ___

3.2. To attend a MSc degree: 1st year ___ 2nd year ___ 3º year ___

3.3. To attend a PhD degree: 1st year ___ 2nd year ___ 3º year ___

3.4. Others ____________________.

3.4.1. Specify ____

4. Education area/ Course: ____

5. Have you ever observed benthic macroinvertebrates with a stereomicroscope? Yes ___   No ___

5.1. If so, please, indicate in what context.

____

6. Have you some experience in the macroinvertebrates identification, by using dichotomous keys? Yes ___   No ___

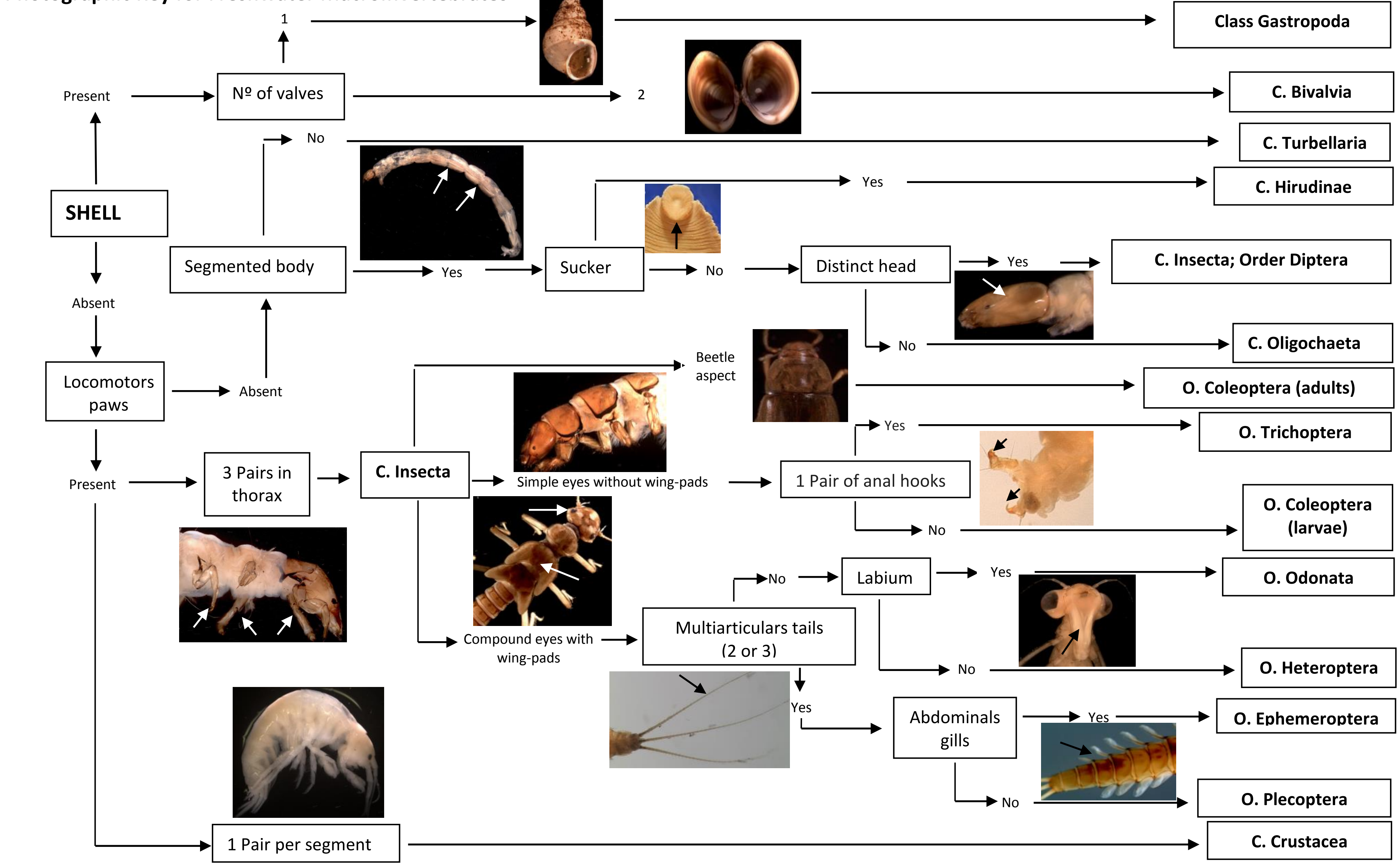
6.1. If so, to what taxonomic level have you already identified?

____

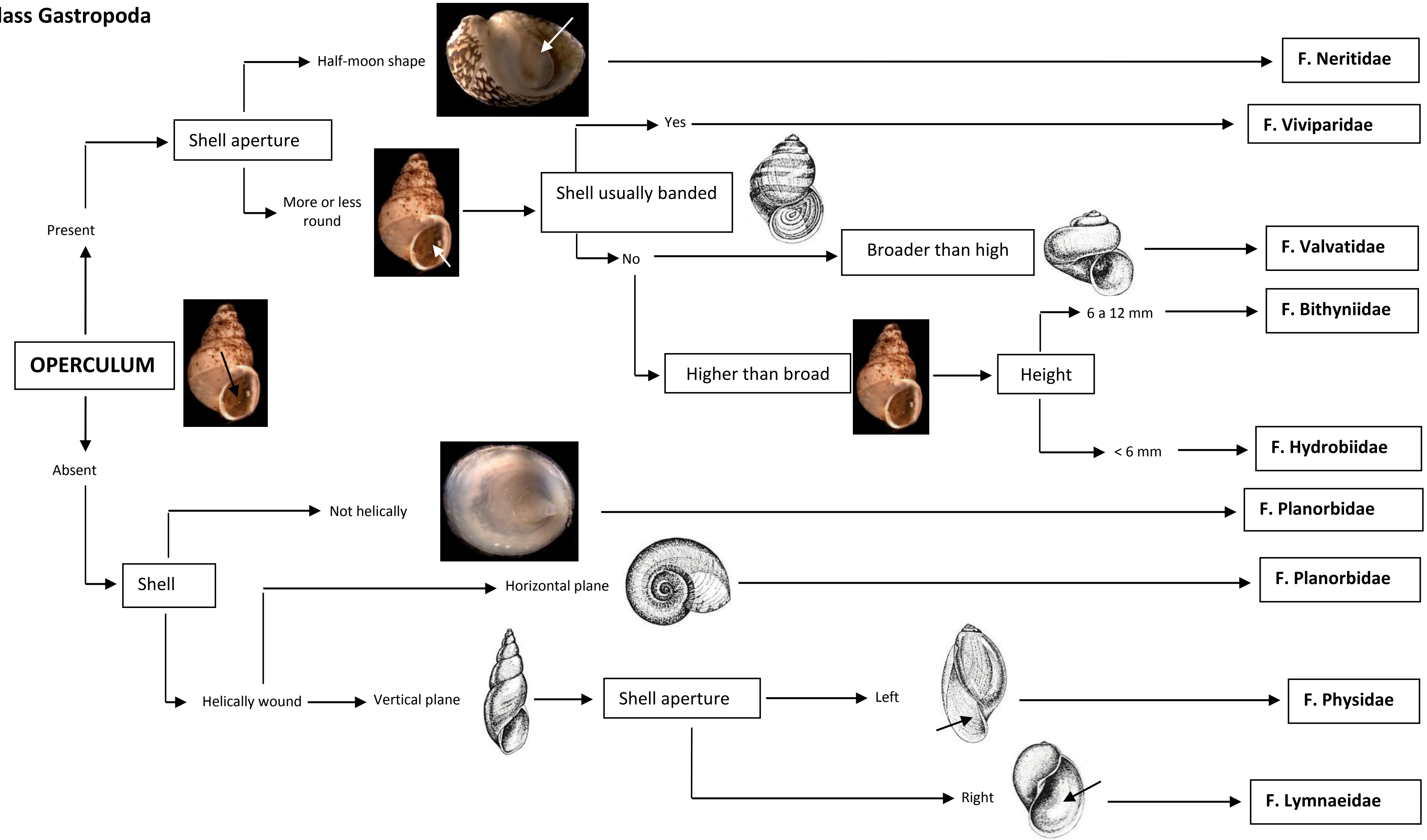
Part II

Organism 1	**Organism** 2	**Organism** 3
1. Identify the organism: ______________________ 1.1. If you didn't identify, indicate the reasons. ____ ____ ____ 2. Did you use the photographic dichotomous key, in a sequentially way, with ease/difficulty (scratch the word which is not applicable). Indicate the reasons. ____ ____ ____ 3. Did you had more difficulty in some steps of the photographic dichotomous key? Yes ___ No ___ 3.1. If you answered yes, indicate what steps and corresponding reasons. ____ ____ ____ 4. Did you had other difficulties in the use of the photographic dichotomous key? Yes ___ No ___ 4.1. If you answered yes, indicate which difficulties. ____ ____	1. Identify the organism: ______________________ 1.1. If you didn't identify, indicate the reasons. ____ ____ ____ 2. Did you use the photographic dichotomous key, in a sequentially way, with ease/difficulty (scratch the word which is not applicable). Indicate the reasons. ____ ____ ____ 3. Did you had more difficulty in some steps of the photographic dichotomous key? Yes ___ No ___ 3.1. If you answered yes, indicate what steps and corresponding reasons. ____ ____ ____ 4. Did you had other difficulties in the use of the photographic dichotomous key? Yes ___ No ___ 4.1. If you answered yes, indicate which difficulties. ____ ____	1. Identify the organism: ______________________ 1.1. If you didn't identify, indicate the reasons. ____ ____ ____ 2. Did you use the photographic dichotomous key, in a sequentially way, with ease/difficulty (scratch the word which is not applicable). Indicate the reasons. ____ ____ ____ 3. Did you had more difficulty in some steps of the photographic dichotomous key? Yes ___ No ___ 3.1. If you answered yes, indicate what steps and corresponding reasons. ____ ____ ____ 4. Did you had other difficulties in the use of the photographic dichotomous key? Yes ___ No ___ 4.1. If you answered yes, indicate which difficulties. ____ ____

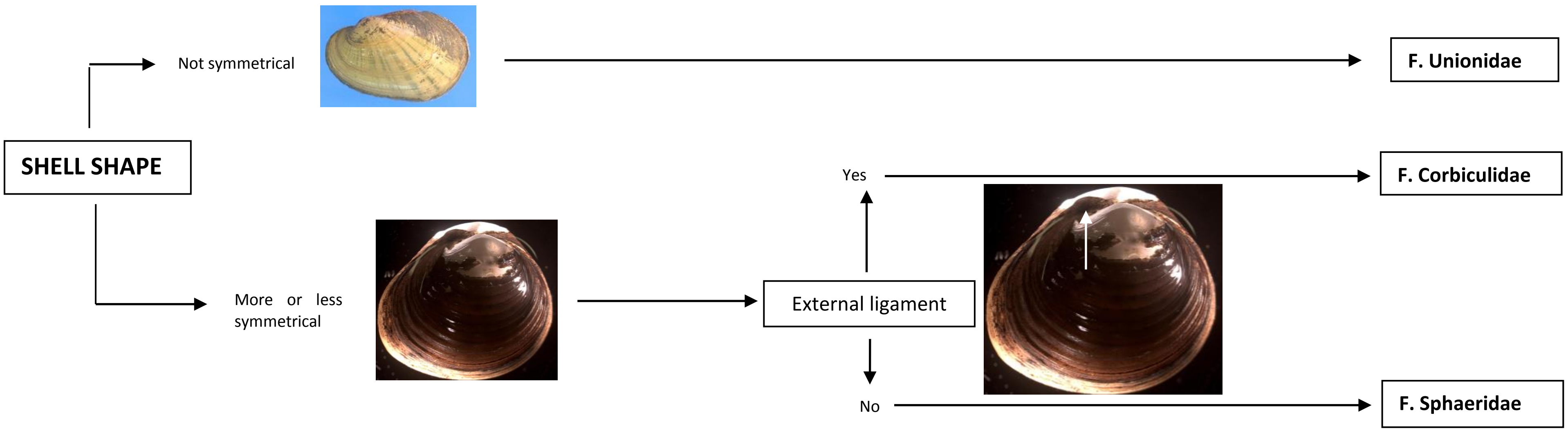
Photographic Key for Freshwater Macroinvertebrates



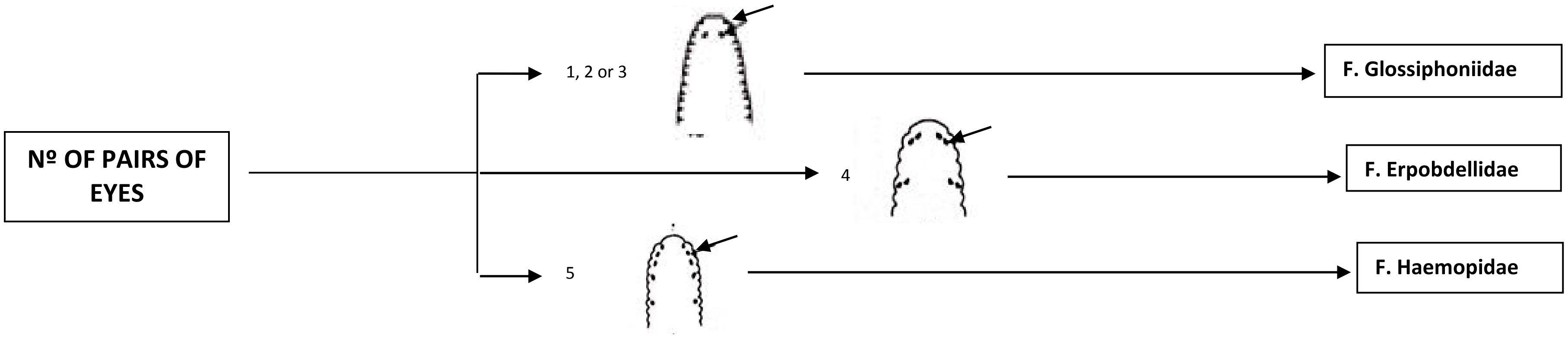
Class Gastropoda



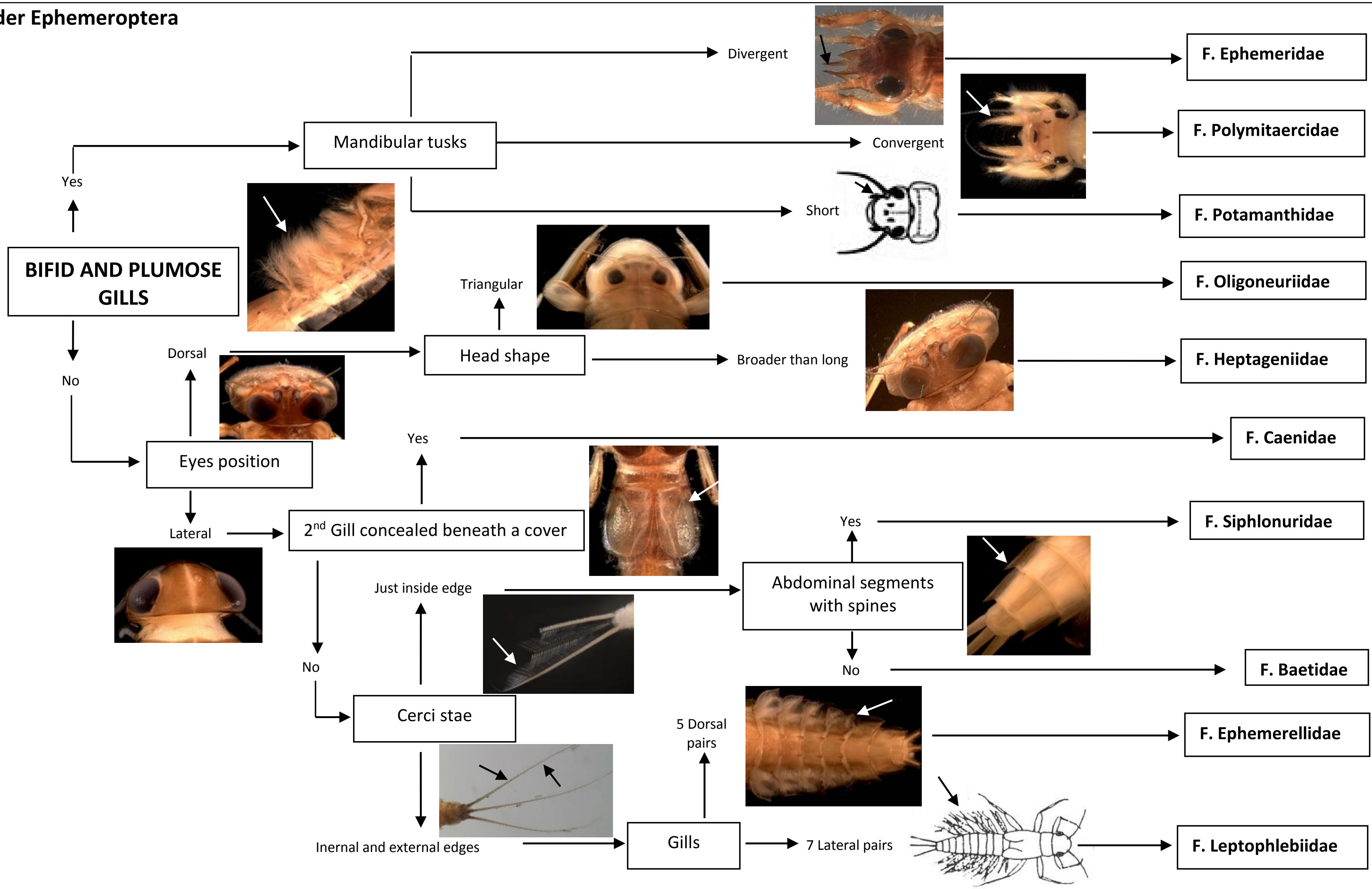
Class Bivalvia



Class Hirudinea



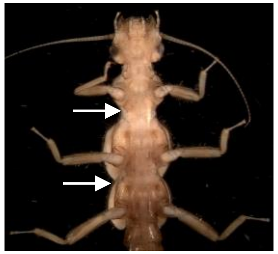
Order Ephemeroptera



Order Plecoptera

LAST SEGMENT OF MAXILLARY PALPS POINTED

Yes → Lack of thoracic gills



No → **F. Perlidae**

Yes → Last segment of maxillary palp

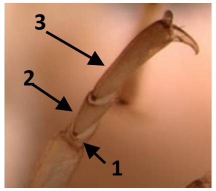
With = 1/4

→ **F. Chloroperlidae**

Largura = 1/2

→ **F. Perlodidae**

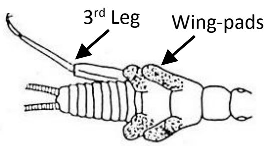
No → Tarse 1 ‹ 2 ‹ 3



Yes → **F. Taeniopterygidae**

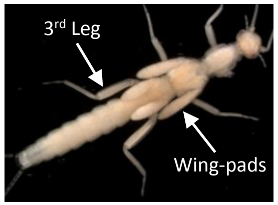
No → 3^{rd} Leg size + Wing-pads

Long + Divergents



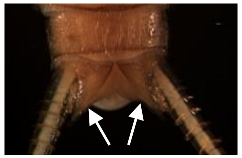
→ **F. Nemouridae**

Short + Paralels



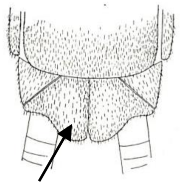
→ Sub-anal plates

Longer than wider



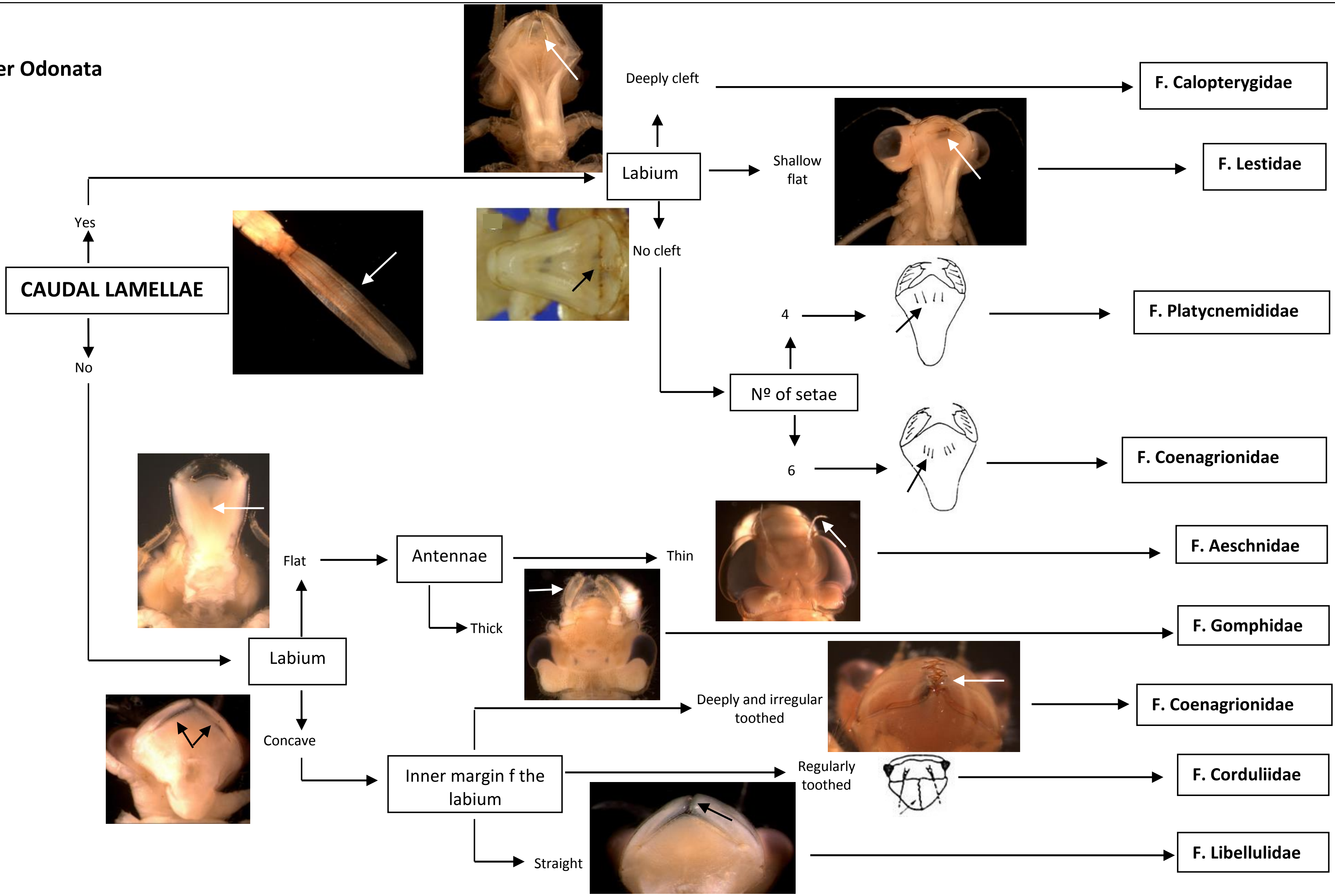
→ **F. Leuctridae**

Wider than long

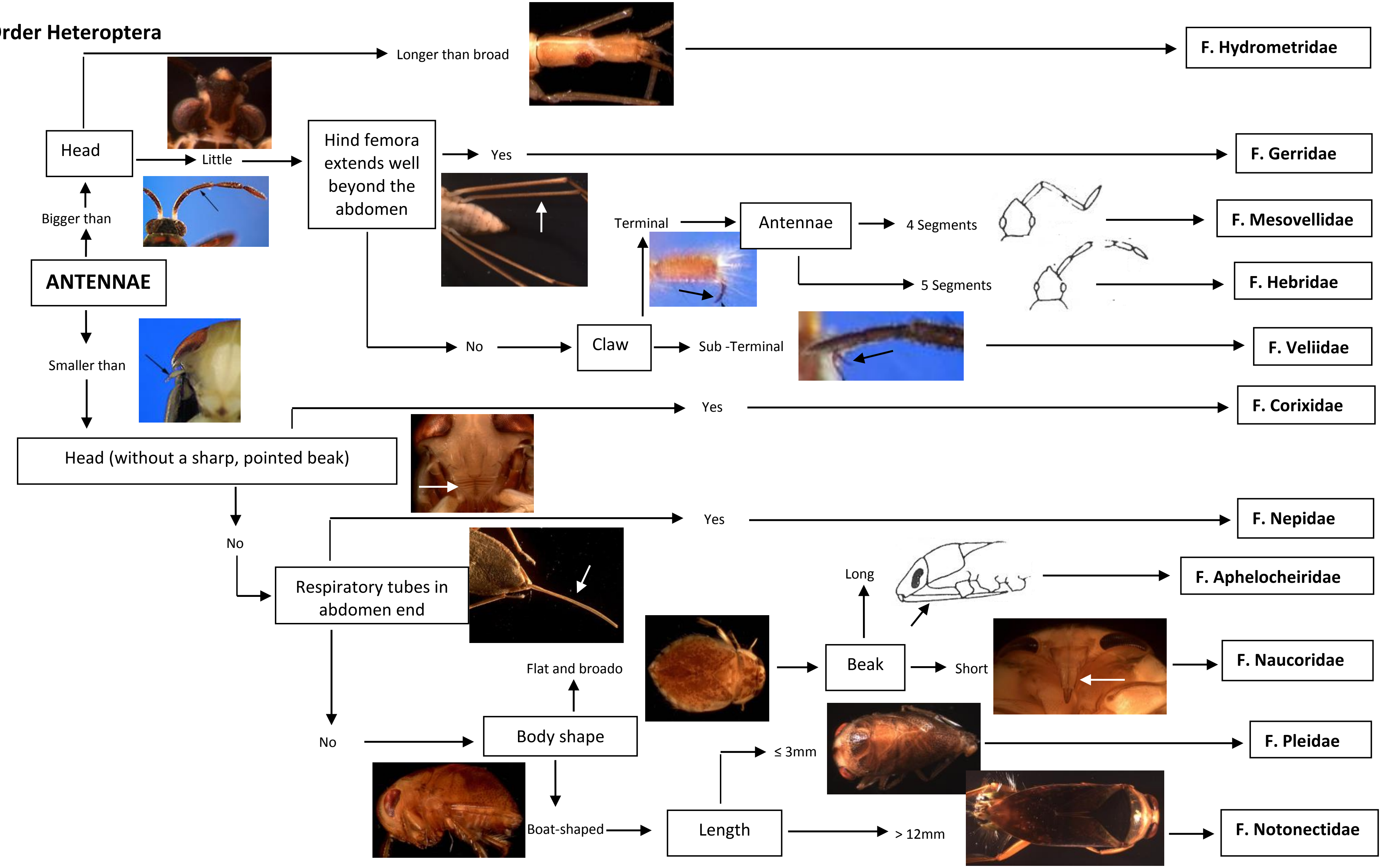


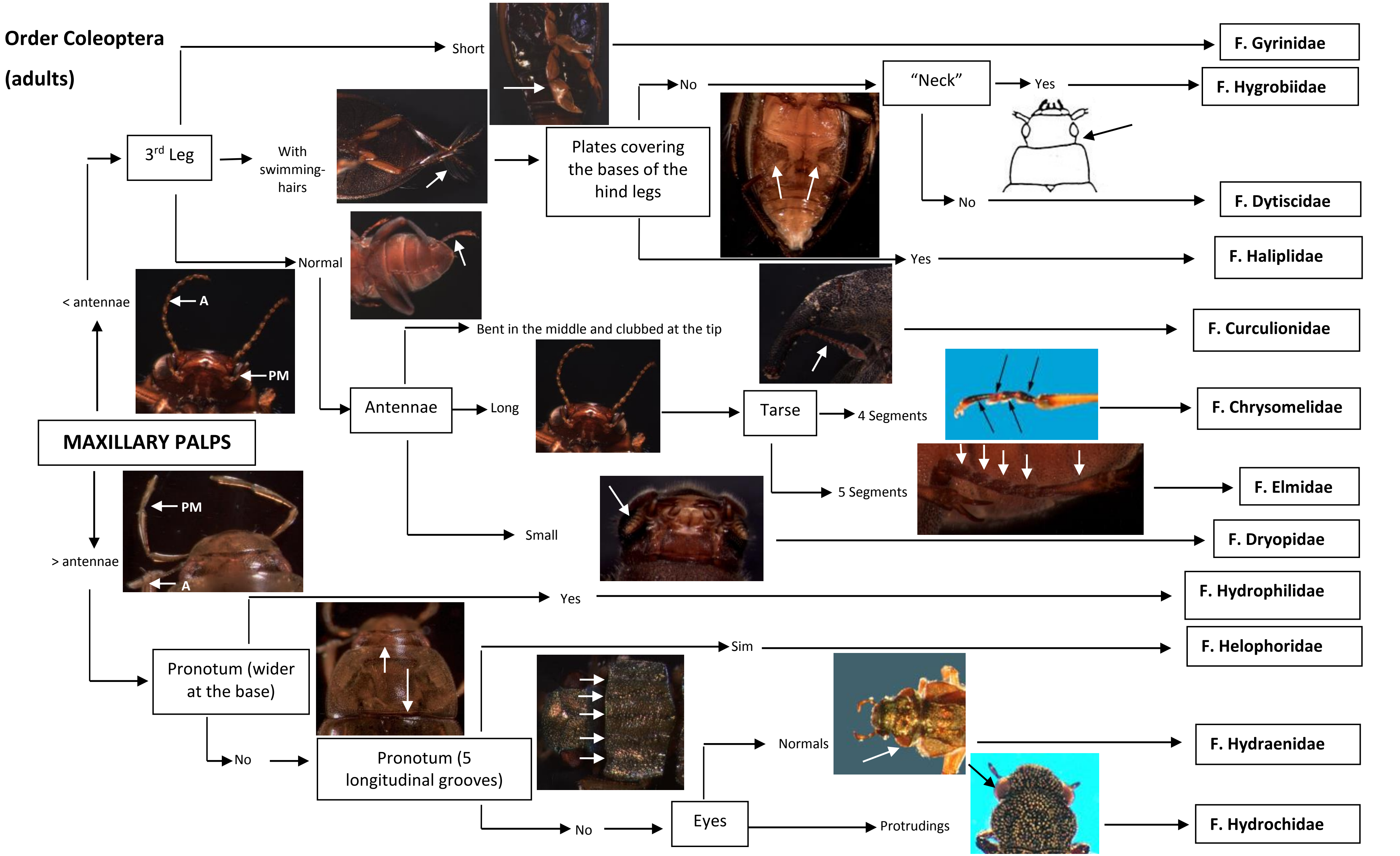
→ **F. Capniidae**

Order Odonata



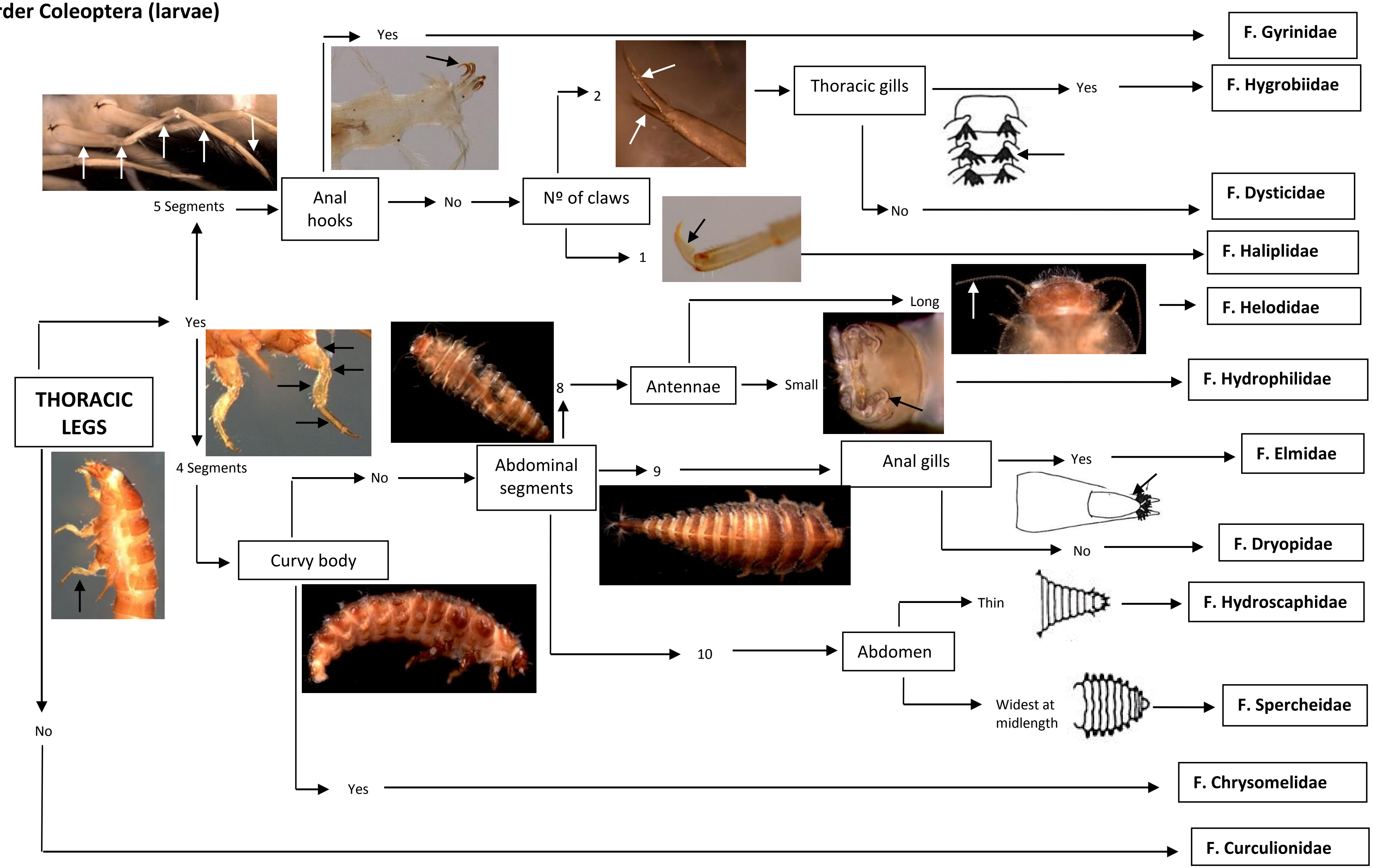
Order Heteroptera



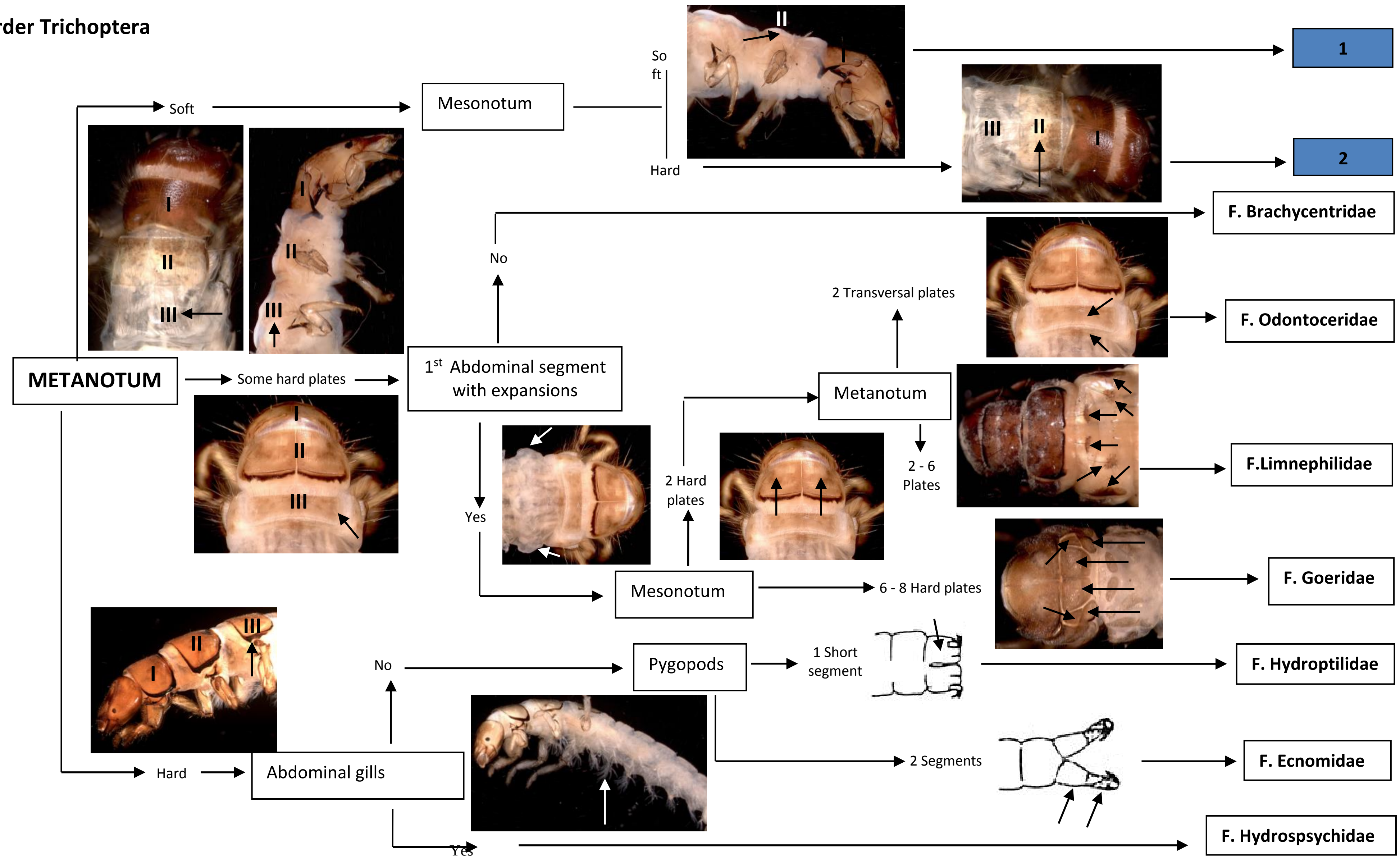


Order Coleoptera
(adults)
MAXILLARY PALPS
< antennae
A
PM
> antennae
3rd Leg
Short
With swimming-hairs
Normal
Plates covering the bases of the hind legs
No
Yes
"Neck"
Yes
No
F. Gyrinidae
F. Hygrobiidae
F. Dytiscidae
F. Haliplidae
Antennae
Bent in the middle and clubbed at the tip
F. Curculionidae
Long
Tarse
4 Segments
F. Chrysomelidae
5 Segments
F. Elmidae
Small
F. Dryopidae
Pronotum (wider at the base)
Yes
F. Hydrophilidae
No
Pronotum (5 longitudinal grooves)
Sim
F. Helophoridae
No
Eyes
Normals
F. Hydraenidae
Protrudings
F. Hydrochidae

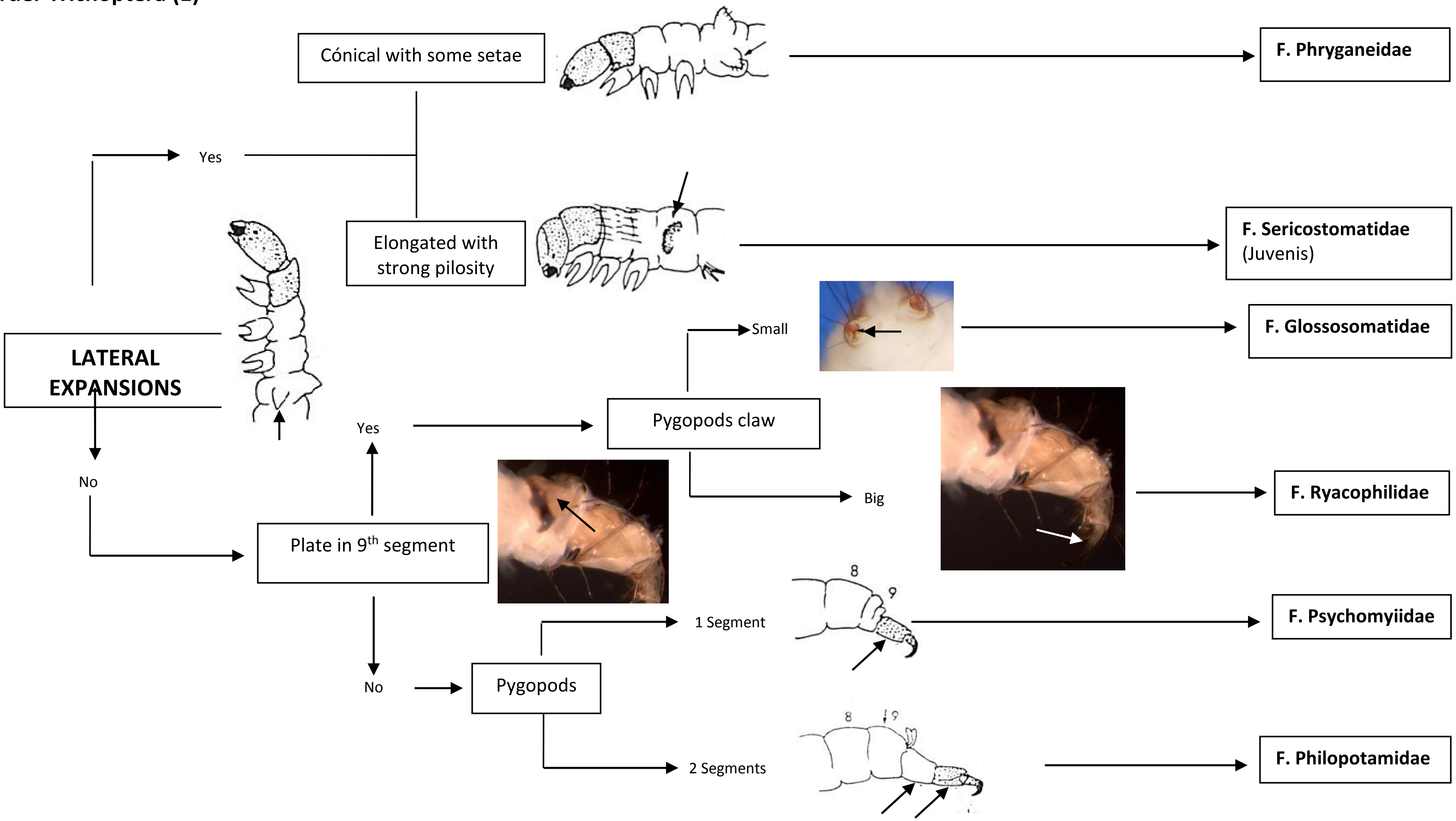
Order Coleoptera (larvae)



Order Trichoptera



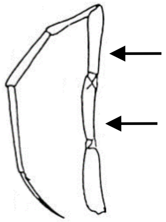
Order Trichoptera (1)



Order Trichoptera (2)

3rd LEG FEMORA

2 Parts



→ **F. Leptoceridae**

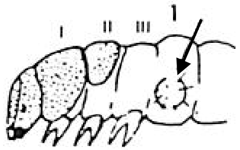
1 Part

→ Larvae case

Conic

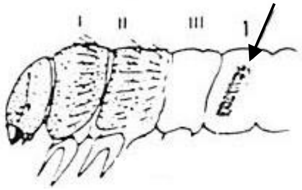
→ Lateral expansions

Rounded with some setae



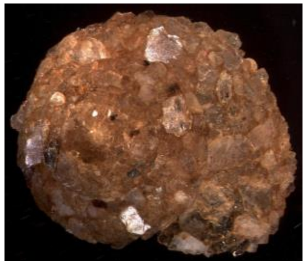
→ **F. Beraeidae**

Elongated with dense pilosity

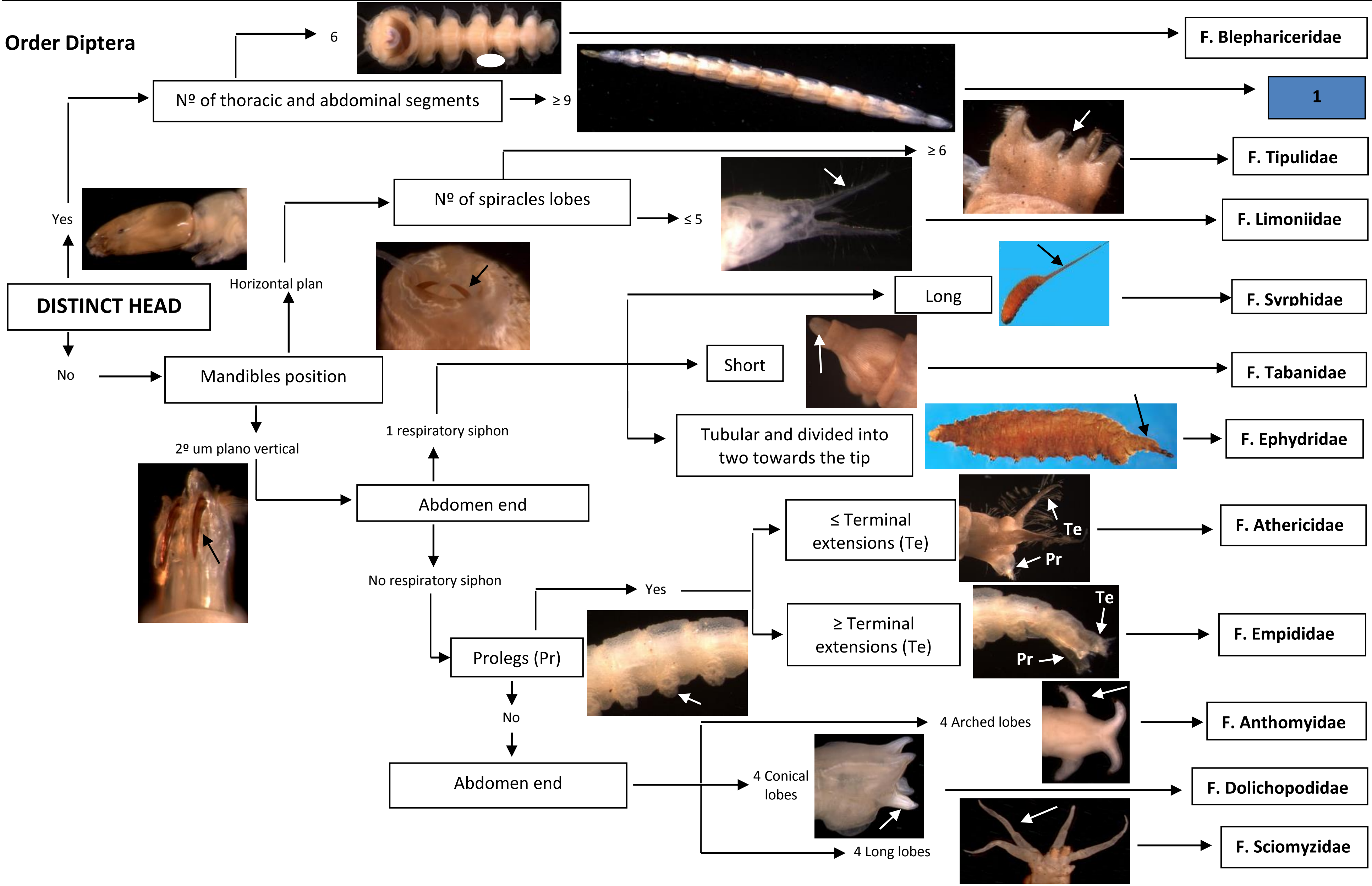


→ **F. Sericostomatidae**

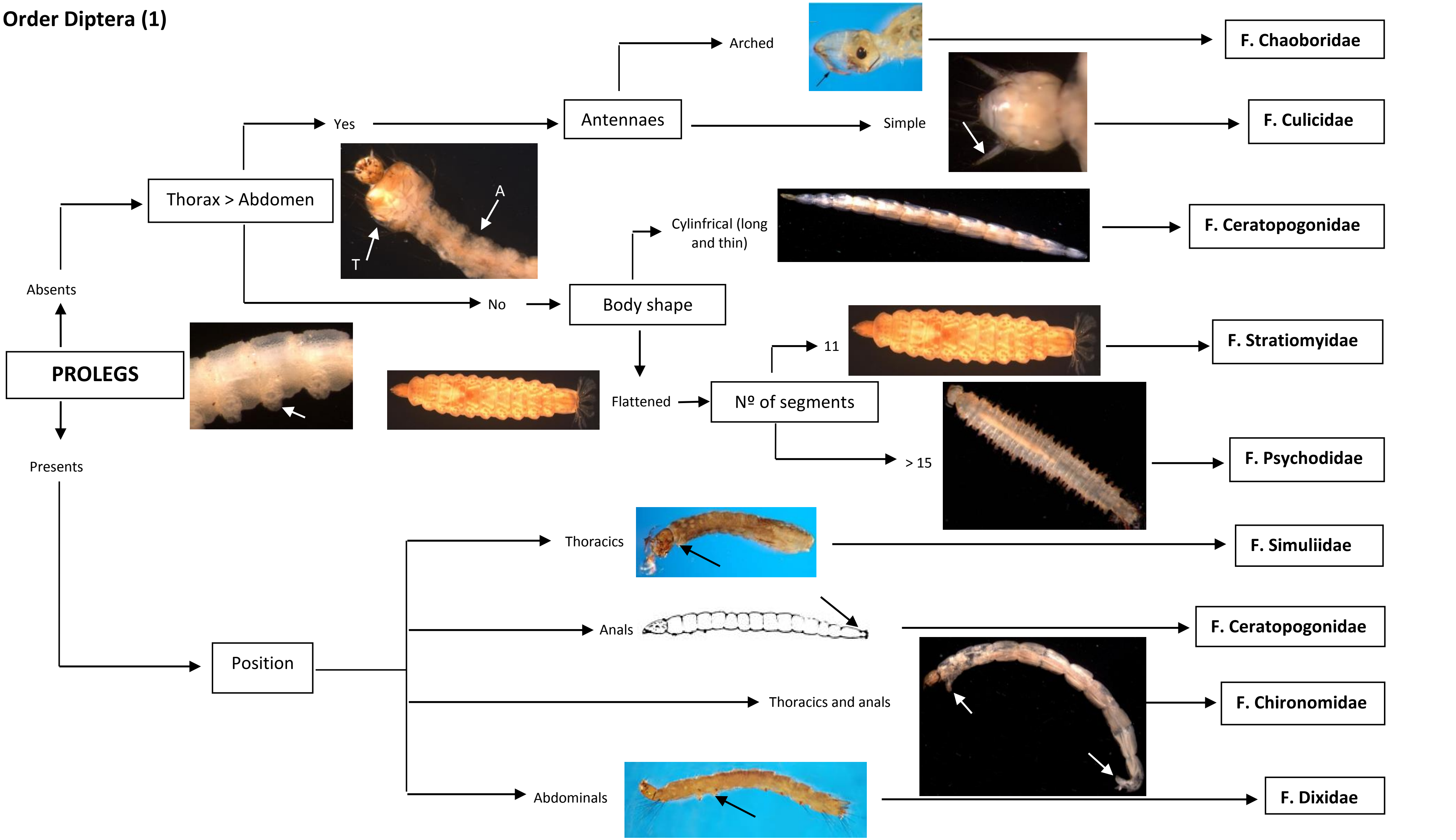
Helical



→ **F. Helicopsychidae**


Order Diptera
DISTINCT HEAD
Yes
No
Nº of thoracic and abdominal segments
6
≥ 9
F. Blephariceridae
1
Nº of spiracles lobes
≥ 6
≤ 5
F. Tipulidae
F. Limoniidae
Horizontal plan
Mandibles position
2º um plano vertical
Abdomen end
1 respiratory siphon
Long
Short
Tubular and divided into two towards the tip
F. Syrphidae
F. Tabanidae
F. Ephydridae
No respiratory siphon
Prolegs (Pr)
Yes
≤ Terminal extensions (Te)
≥ Terminal extensions (Te)
Te
Pr
F. Athericidae
F. Empididae
No
Abdomen end
4 Arched lobes
4 Conical lobes
4 Long lobes
F. Anthomyidae
F. Dolichopodidae
F. Sciomyzidae

Order Diptera (1)

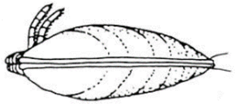


Class Crustacea

CARAPACE

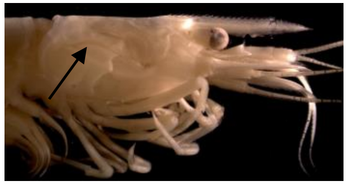
Yes

In the form of 2 valves



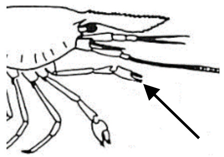
F. Limnadiidae

Covers the head and thorax



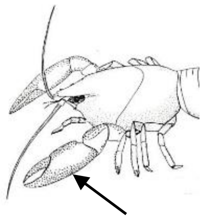
"Pincers"

Smalls

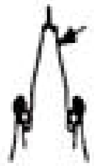


F. Atyidae

Strongs



Rostrum convergent margins



Yes

F. Cambaridae

No

F. Astacidae

No

Body shape

Flatenned from side to side

F. Gammaridae

Flattened from top to bottom

F. Asellidae

CAPÍTULO VI

Considerações Finais

Desde a publicação da Diretiva-Quadro da Água (DQA), em 2000, muito trabalho foi feito, com o objetivo de aplicar e desenvolver esta diretiva em ecossistemas de água doce. No entanto, a DQA propôs, até ao final de 2015 (data adiada para 2027), não só estabelecer a classificação das massas de água, como também recuperar as ribeiras e rios classificados com um estado ecológico inferior a Bom (CE, 2000).

A implementação da DQA tem impulsionado, por um lado, o desenvolvimento de uma série de conceitos comuns, terminologias e ferramentas, e por outro o desenvolvimento de novos índices (Pranovi, Da Ponte & Torricelli, 2007). Membros da UE, como a Europa do norte e do centro, têm dedicado muito do seu trabalho à otimização e harmonização de técnicas e metodologias, devido às suas eco regiões semelhantes; enquanto o sul da Europa, com diferentes eco regiões e consequentemente diferentes ecossistemas, tenta acompanhar e adaptar as metodologias da DQA. Apesar destes esforços, por exemplo, em Portugal e na Grécia, não há planos de gestão das bacias hidrográficas adotados ou comunicados à Comissão Europeia (Perni & Martinez-Paz, 2013), independentemente do trabalho desenvolvido por instituições nacionais.

Na DQA, o estado ecológico das águas de superfície é definida com base num esquema de avaliação integrada, que combina elementos hidromorfológicos, físico-químicos e biológico. Cada elemento de qualidade, por si só, dá informações específicas sobre o estado dos sistemas aquáticos. Por exemplo, os elementos físico-químicos fornecem informações sobre os efeitos de *stress* nos processos físicos e químicos do ecossistema, mas não esclarecem como é que podem afetar as comunidades biológicas. Por outro lado, os elementos de qualidade biológica refletem os efeitos de poluentes sobre a biota sem esclarecer os agentes de *stress* ambientais responsáveis pelas alterações da comunidade. Apesar de permitir uma visão holística do funcionamento dos ecossistemas, as metodologias de avaliação adotadas pela DQA não permitem estabelecer relações de causa - efeito entre os fatores de *stress* e mudanças nas comunidades aquáticas, que é crucial para a gestão dos ecossistemas de água doce (Serpa et al., 2014). Neste sentido, o presente trabalho foi desenvolvido com o intuito de avaliar a qualidade ecológica de sistemas lóticos selecionados, tendo em conta a forma como múltiplos agentes de *stress* (físicos, químicos e biológicos) afetam a estrutura das comunidades de macroinvertebrados bentónicos, através da utilização da análise multivariável e de metodologias definidas pela DQA. Assim, pretendeu-se discutir se estas últimas oferecem a resolução necessária para distinguir impactos de agentes de *stress* que não a poluição difusa, para a qual a DQA terá sido, no geral, aplicada.

Em suma, os resultados obtidos evidenciaram que o uso de índices bióticos, apesar de ser a metodologia adotada, no âmbito da DQA, não é tão discriminativo como a análise da estrutura da comunidade de macroinvertebrados bentónicos. A análise multivariável é, muitas vezes, aplicada para caracterizar e avaliar a qualidade de água doce, porque é útil para verificar as variações temporais e espaciais causadas por fatores naturais e antrópicos (Varol, Gokot, Bekleyen, & Sen, 2012). Um dos problemas limitantes ao uso desta última abordagem foi a baixa abundância de macroinvertebrados colhidos durante o período de amostragem realizada em S. Domingos (capítulo III), não tornando possível a sua aplicação.

A implementação da DQA também é muito complexa, pois exige um trabalho muito especializado para provar, reunir e integrar informações de diferentes fontes, incluindo as comunidades biológicas que habitam cada local monitorizado. Para se avaliar o impacto de perturbações nos ecossistemas lóticos utilizam-se índices de diversidade e bióticos que facilitam a interpretação de mudanças na comunidade de macroinvertebrados bentónicos (Gray & Delaney, 2010). No entanto, Washington (1984) fornece uma excelente revisão acerca destes índices, demostrando que estes são, muitas vezes, mal utilizados por serem i) especializados em comparar condições ambientais associadas apenas com un determinado tipo de poluição, a poluição orgânica; ii) limitados a áreas geográficas específicas; e iii) terem uma relevância ecológica limitada, não separando alterações ambientais que ocorrem naturalmente no meio aquático das que estão relacionadas com os vários tipos de *stress* antrópicos. Consequentemente, ao serem utilizados fora do contexto mais apropriado, os índices bióticos podem não descrever, com precisão, as condições ambientais presentes no meio que pretendem avaliar. Contrariamente, os índices de diversidade não são específicos para uma determinada perturbação, mas para avaliar o estado geral do sistema. (Washington, 1984).

Não há "índices perfeitos " nem metodologias perfeitas para avaliar as diferenças entre as várias comunidades biológicas que existem no meio aquático (Chapman, 2011). Na verdade, é necessária mais investigação para resolver incertezas que persistem, desenvolvendo uma abordagem integrada com o levantamento de outros elementos biológicos e com ensaios toxicológicos agudos e/ou crónicos com organismos de diferentes níveis tróficos. Estes ensaios podem melhorar sensivelmente o conhecimento sobre os efeitos biológicos de vários agentes antrópicos, melhorando, assim, a base científica de avaliação de risco ecológico (Serpa et al., 2014).

O desejo de restaurar a biodiversidade, em rios que foram degradados por vários fatores, como por exemplo o uso excessivo de fertilizantes, a construção de barragens, a introdução de espécies, tem vindo a aumentar e o envolvimento das partes interessadas e da sociedade civil é muito importante na tomada de decisões para a resolução desses problemas. Assim, com o planeamento de iniciativas de conservação, através de parcerias, os profissionais e outros cidadãos podem melhorar a proteção dos ecossistemas locais, através da partilha de recursos, promovendo um entendimento comum e, finalmente, trabalhar em conjunto para influenciar, efetivamente, a política ambiental (Norton, 2003). Neste contexto, é importante a construção de ferramentas, visualmente atrativas, fáceis de interpretar e de compreender, disponíveis para qualquer cidadão. Uma dessas ferramentas pode ser o desenvolvimento de uma chave dicotómica, fotográfica e simplificada, de identificação de macroinvertebrados bentónicos existentes em Portugal destinada para qualquer público, com pouco conhecimento científico nesta área (capítulo V). Uma estratégia científica para a comunicação e transferência de conhecimento científico pode provocar mudanças no comportamento/atitude da sociedade perante a conservação de ecossistemas dulçaquícolas.

Referências bibliográficas

CE (2000). Diretiva 2000/60/CE de 23 de Outubro. (2000). Jornal Oficial das Comunidades Europeias, L327. Parlamento Europeu e do Conselho, Portugal.

Chapman, P.M. (2011). Indices: attractive delusions. *Integrated Environmental Assessment and Management*, 3 (7), 313.

Gray, N.F., & Delaney, E. (2010). Measuring community response of bentic macroinvertebrates in an erosional river impacted by acid mine drainage by use of a simple model. *Ecological Indicators*, 10, 668-675.

Norton, B. (2003). *Searching for sustainability: interdisciplinary essays in the philosophy of conservation biology*. Cambridge, UK: Cambridge University Press.

Perni, A., & Martinez-Paz, J.M. (2013). A participatory approach for selecting cost-effective measures in the WFD context: The Mar Menor (SE Spain). *The Science of the Total Environment*, 458-460:303-11.

Pranovi, F., Da Ponte, F., & Torricelli, P. (2007). Application of biotic indices and relationship with structural and functional features of macrobenthic community in the lagoon of Venice: an example over a long time series of data. *Marine Pollution Bulletin*, 54: 1607–1618.

Serpa, D., Keizer, J.J., Gonçalves, F., Cassidy, J., Cerqueira, M., Cuco, A., Silva, V., & Abrantes, N. (2014). Assessment of river water quality using an integrated physicochemical, biological and ecotoxicological approach. *Environmental Science: Processes & Impacts*, 16, 1434–1444.

Varol, M., Gökot, B., Bekleyen, A., & Sen, B. (2012). Spatial and temporal variations in surface water quality of the dam reservoirs in the Tigris River basin, Turkey. *Catena*, 92, 11-21.

Washington, H.G. (1984). Diversity, biotic and similarity indices: A review with special relevance to aquatic ecosystems. Water Res., 18, 653–694.